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NORMAL AND PATHOLOGIC PROLIFERATION IN THE BREAST WITH SPECIAL REFERENCE TO CYSTIC DISEASE

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Although the development of the female breast has been exhaustively described by many authors, the light that normal growth throws on the pathologic lesions of later life has been insufficiently appreciated. A technic recently published by me in collaboration with Holly [1] makes complete examination of the whole breast a relatively easy matter. With the use of this technic, facts emerge clearly whose significance was difficult to appreciate with piecemeal methods alone.

The results given in this paper were obtained chiefly by study of serial sections made with the slicer. Paraffin sections were also made, but it was found that the information they gave was meager compared with that obtained from the slicer sections viewed under the dissecting microscope. Reconstruction of the series was done by means of photographs. When it was desired to trace a duct or a group of ducts, photographs were made of both sides of each section in the series. In this way the three dimensional picture seen under the dissecting microscope was translated into a two dimensional record.

OBSERVATIONS

The Adolescent Breast.—The description given here is based on the breasts of 6 girls. Three were 13 years old, 2 were aged 12, and 1 was 10. One of those aged 13 had a cardiac disease. Four others died of acute disease after a brief illness. One died as a consequence of the administration of an anesthetic. The most detailed observations were carried out on a girl of 13, who died of acute rheumatic pneumonia (breast 3). She had been in ill health for a month but was acutely ill only two days. The menses were not established. At autopsy, two hours after death, the breasts and the uterus appeared normal. The ovaries showed numbers of small follicular cysts—a normal finding at

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1. Ingleby, H., and Holly, C.: J. Tech. Methods **19**:93, 1939.

this age. There was no corpus luteum. Microscopically the endometrium showed a normal degree of proliferation, characteristic of estrogenic stimulation.

The adolescent breast consists only of ducts. There are no lobules. The ducts branch along their whole course from beneath the nipple to the muscle. In the tissues seen under the dissecting microscope the first thing that attracts attention is the club-shaped swelling of the majority of the terminal ducts (fig. 1 *A*). A few of the growing ducts are round —this is mostly an earlier stage—and not infrequently, where a duct is about to bifurcate, a kidney-shaped structure is seen. These appearances have been described by other authors, of course, but it comes as a surprise to see how closely these formations resemble bunches of cysts (figs. 1 *A*, 3 *A* and 7 *A*).

A point that emerges very clearly in the sections of breasts 3, 5 and 6 is the irregularity of development in different parts of the breast (fig. 1 *B*). The nodule in the illustration is not an artefact; it extends through some 10 sections in the series. The branches have subdivided to a much greater extent than elsewhere in the breast. It is also to be noticed that development is more advanced in the deeper portions of the breast, toward the muscle, than near the nipple. This may be connected with the very rich blood supply from twigs reaching the breast via the pectoral muscle. These branches were accurately described by Cooper[2] and are easily seen in our sections. They anastomose freely with branches from superficial vessels.

Even more interesting is the irregularity presented in case 6. This breast was from a well developed Negro girl of 12 who died of acute ulcerative colitis after an illness of only six days. She had never menstruated. The ovaries showed the usual small follicular cysts, but on the surface of the right ovary was a small scar, as though ovulation had taken place. Microscopically the right ovary, besides its developing follicles with proliferating granulosa, showed a corpus albicans and a small patch of degenerating lutein cells. The breast in most places presented ducts with stumpy branches typical of early adolescence. The branches were small, but differentiation was more advanced than in the breast from the other 12 year old girl. In one area near the lower edge of the breast there were a number of lobules. The general contour of these lobules was like that of the adult breast, but the ductules of which they were composed were wider, more irregular and stumpy as compared with those of the adult.

Breast 5 was from a Negro girl aged 13 who died of septicemia after seven days' illness. Onset of menstruation occurred nine months before

2. Cooper, A. P.: On the Anatomy of the Breast, London, Longman [and others], 1840.

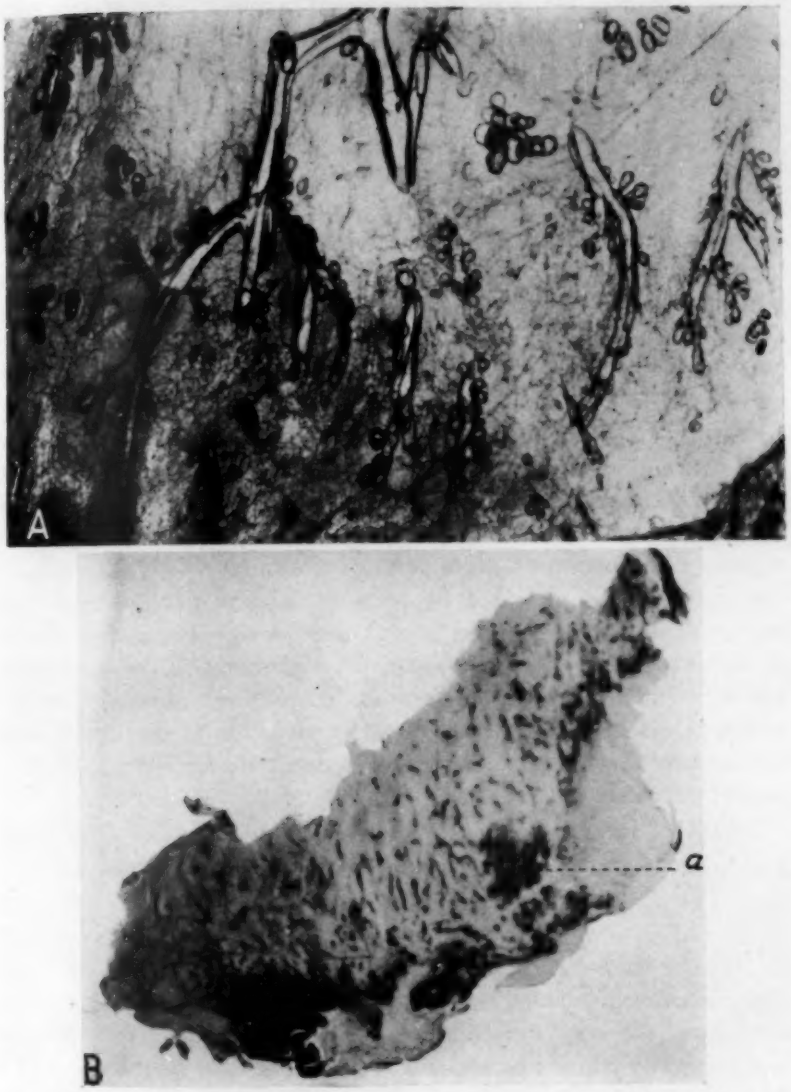

Fig. 1 (breast 3).—Normal adolescent breast from a girl aged 13, who died of rheumatic pneumonia two days after the onset of acute illness. In *A* the terminal ducts show rounded or club-shaped swellings. Ridges are visible in some of the ducts. *B* is a section through the whole breast. The development is not uniform. At *a* there is an area of more advanced differentiation.

These and subsequent illustrations are from sections made with the slicer unless otherwise stated and photographed with extension tubes for enlarging.

death. The periods were regular, occurring every twenty-eight days, the last one two weeks before death. The findings in the uterus and ovaries corresponded with the menstrual history. Part of this breast showed the same general pattern as the others, although the branching was finer. But elsewhere many lobules of a more or less adult type were scattered through the tissue. In fact, all stages of development were represented, except of course those belonging to the second part of the adult cycle.

Breast 4, also from a girl of 13 years, showed ducts which were of the same pattern but smaller and more widely separated than those in breast 3. The development was more advanced in some parts than in others but to a less striking extent, and individual units appeared more uniform. This child died of congestive heart failure after an illness of two and one-half months. There seems to be retardation of growth in the breast, which would account for the comparative uniformity. Breast 2 (fig. 2 *A*), from a girl aged 12, represents an earlier stage. The whole organ is much smaller and the branches less developed. Proliferation of endometrium had scarcely begun. No striking variation in differentiation could be expected at this stage, but there was some difference in the extent of branching in different sections. Breast 1 (fig. 2 *B*), from a girl aged 10, presents an early stage of budding-off of ducts. The breast was of course very small. The general pattern is similar to that seen in the breasts from the other girls, but the branches are short and stumpy. Here again development does not proceed everywhere at the same pace. Cystlike dilatation of portions of ducts is seen here and there. This is not an abnormality but is a phase of normal development.

Perhaps the most intriguing feature in our sections were the prominent spurs or ridges seen in the long axis in practically all the ducts (figs. 1 *A* and 3 *A*), although not usually in the end bulbs. The ridges are elevations projecting into the lumen of the duct. They consist of the usual two layers of epithelium and periductal connective tissue. There is no distortion. They are as much a part of the wall of a normal duct as are the intervening grooves. They run for long distances. At first glance they might be thought to be due to a folding of the wall of the duct following shrinkage or emptying of the content of the duct. But if the ridges are traced through several sections to their ultimate destination, it is seen that eventually they meet to form the distal boundary of the mouth of a new duct. The significance of the ridges is made clear by a study of the way in which ducts are formed. When observations made on large numbers of sections are put together, the process of duct

formation seems to be as follows: The first change is a proliferation of epithelial cells in the wall of a duct (fig. 3 *B*). If this proliferation were uniform, all that would happen would be that the duct would become

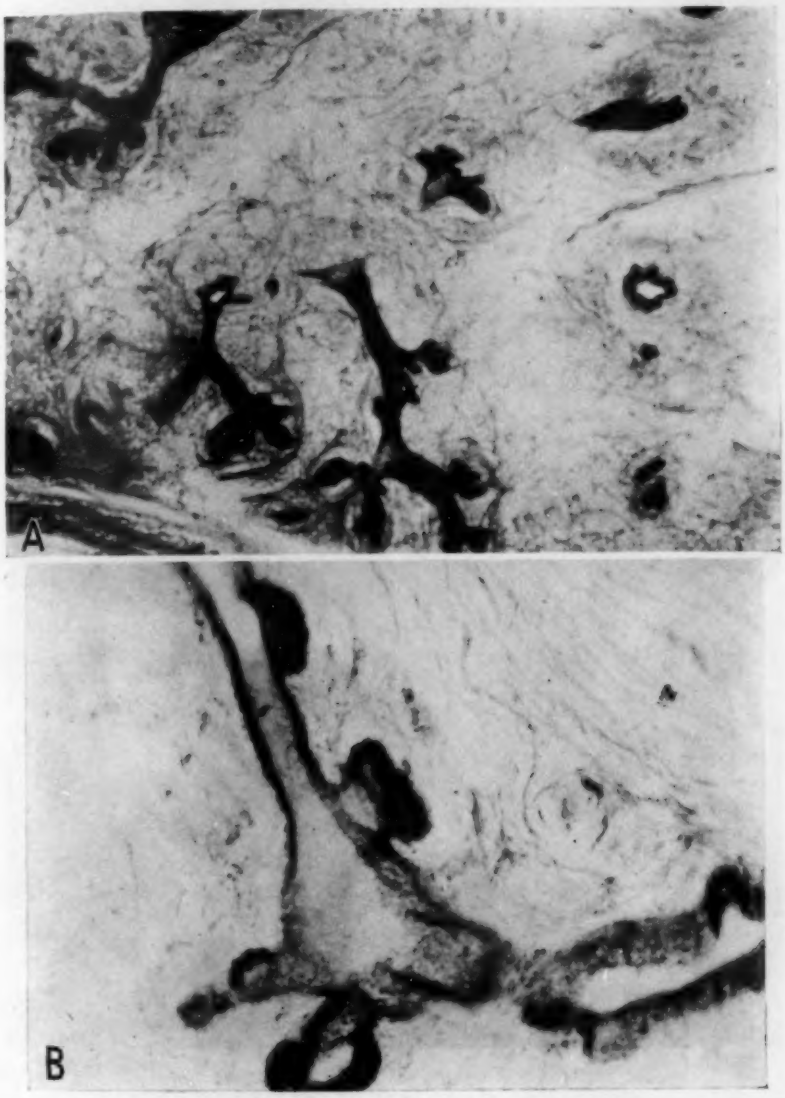

Fig. 2 (breast 2).—*A*, normal adolescent breast from a girl aged 12. *B* (breast 1), photomicrograph (low power) of a paraffin section from the breast of a 10 year old girl, who died as the result of administration of an anesthetic. An early stage of adolescent development is shown. Note the early budding and cystlike dilatations of ducts.

larger and longer. But as the illustration shows, the process is not uniform. The cells proliferate at certain points and grow outward. At first they are seen simply as a clump of cells. Very early a lumen

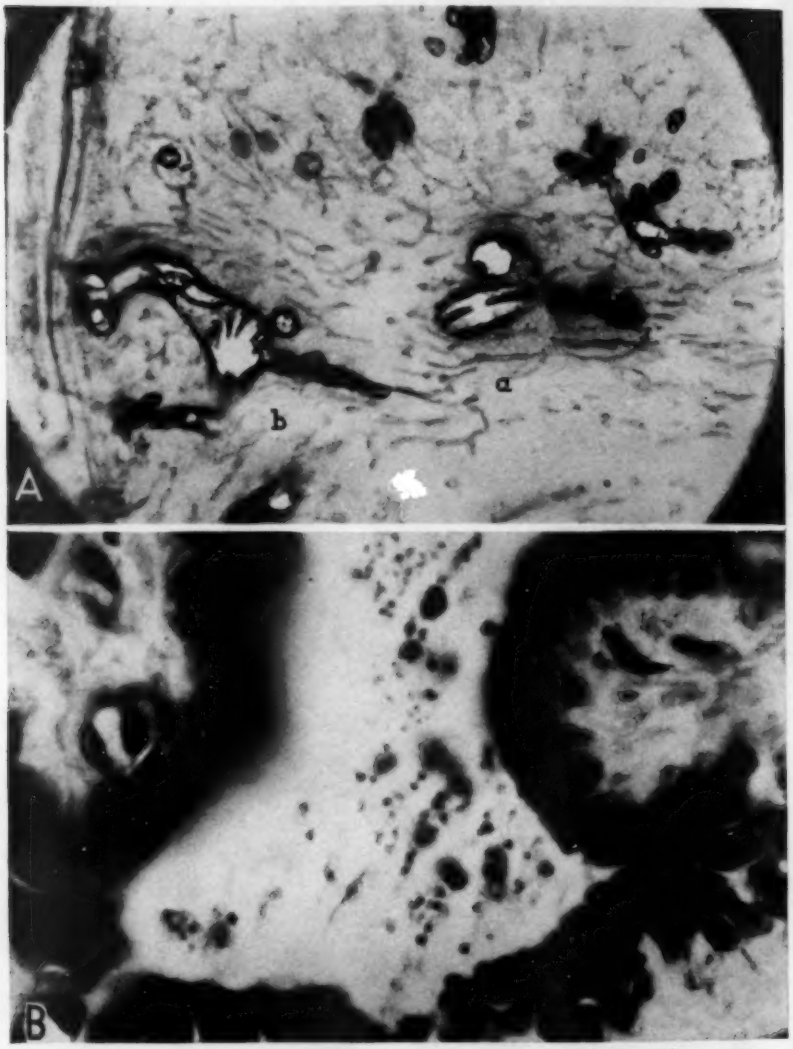

Fig. 3 (breast 3).—Normal adolescent breast from a girl aged 13. At *a* and *b* are ridges projecting into the lumens of ducts *B*, photomicrograph of a paraffin section (oil immersion) to show the earliest stage of duct formation.

appears. The intervening wall which has not proliferated is left behind and becomes a ridge projecting into the lumen. This description applies to the smallest ducts as seen in paraffin sections. But the two-

dimensional view does not give a clear idea of the whole process. Seen in three dimensions, the smallest budding ducts are often round, as has been said. Generally they look like a child's toy balloon on a thick stalk. As these balloon-like objects are followed to the next stage, broad indentations appear in the wall. These represent further branches. A little later the cluster of branches looks like a bunch of cysts. Evidently, the most rapidly growing point is at the distal end of the duct, since these structures elongate, becoming first club shaped and finally taking on the proportions of the adult duct. But elongation must also take place along the whole system of ducts. This becomes strikingly evident when the 10 and 12 year old breasts are compared with the 13 year old breast. In the younger breasts, stubby branches arise very close to each other, and ridges, though present, are less conspicuous. Figure 2 *B* taken from the 10 year old breast makes this clear. Now if, in imagination, these ducts are pulled out to the length of those in the 13 year old breast, the outpouchings at the mouths of the branches would also be elongated and the branches themselves would be further apart. The outpouchings thus form grooves separated by ridges. Beyond the opening of each branch, those particular ridges forming the boundary of the groove unite and disappear.

The connective tissue in young breasts is always well developed. One is apt to think of connective tissue as something which fills the gaps between the ducts and lobules, but of course it is much more than this. It is an integral part of the organ. Adipose tissue, normal in older women, might be considered merely padding, but not the connective tissue. Differentiation of periductal and perilobular connective tissue begins early. Breast 1 shows it to some extent; breast 2, clearly. It is true that there are no lobules at this stage, yet there is a distinct difference between the tissue immediately surrounding the ducts and that farther out. In the 13 year old breasts a clear zone is seen immediately around the ducts. In the normal woman the periductal connective tissue becomes looser and undergoes mucoid degeneration in the second half of the cycle when the epithelial cells proliferate and swell. A similar change probably allows for epithelial proliferation at the time of puberty. No elastic fibers can be seen around the ducts at this time.

It is not surprising that in general pattern the normal prepubertal female breast, the hypertrophied male breast and the pathologic hypertrophied breast of a young woman should be similar, since the condition seen in all three may be produced by estrogenic stimulation. But the

resemblance brought out by serial slicer sections is very striking (figs. 2 *A* and 4 *A* and *B*). All three show the same type of duct growth, and in all three there is failure to produce lobules.

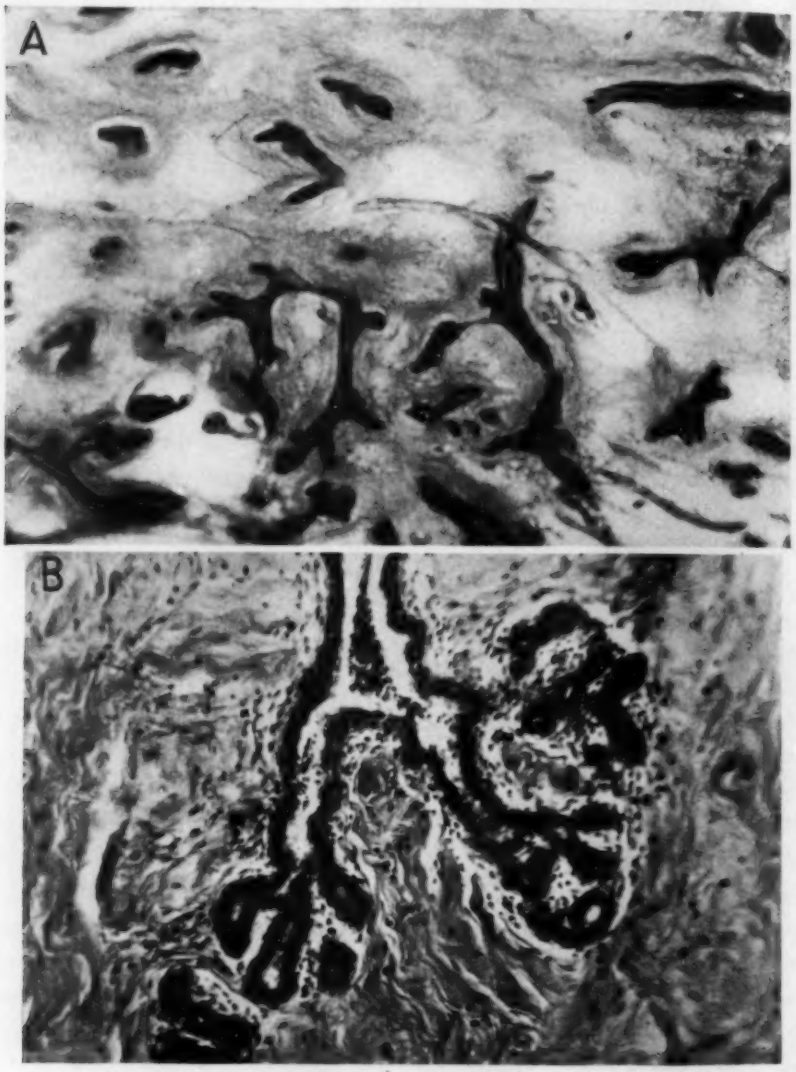

Fig. 4.—*A* (breast 71), hypertrophied breast from a man aged 23 for comparison with *B* in this figure and with *A* in figure 2. *B* (breast 80), photomicrograph (low power) of a paraffin section of a hypertrophied right breast from a woman aged 30, with a history of increasing enlargement of the breast during four years and of pain before menstrual periods.

The Sexual Cycle.—Present knowledge of the sexual cycle is fragmentary, and I shall not attempt to describe it. The pictures will give

some idea of the enormous growth and regression which take place (fig. 5). Without a large number of normal specimens—which I have been unable to obtain—it would be absurd to dogmatize. One point

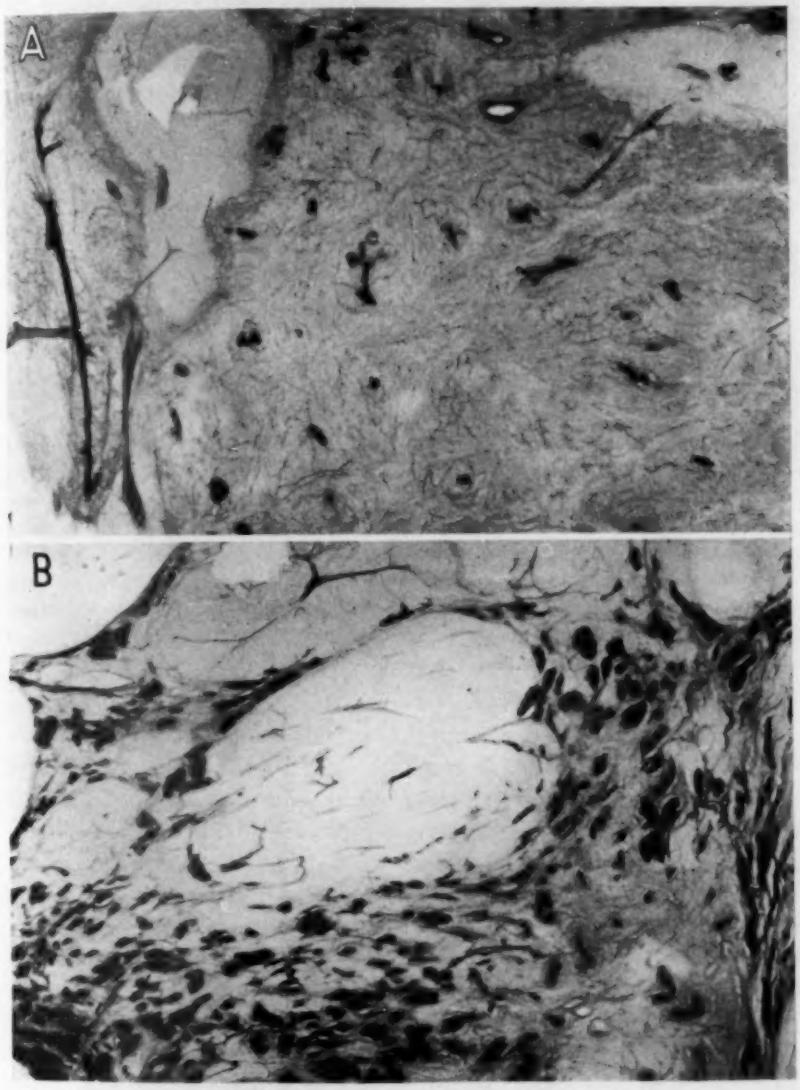

Fig. 5.—*A* (breast 21), an autopsy specimen from a woman aged 20, whose death occurred ten days after the onset of the last menstrual period. *B* (breast 30), normal breast from a woman aged 39 two days after the end of a menstrual period, with the same magnification as for *B*.

emerges: The development and the regression of lobules are not uniform. The irregularity seen in the adolescent is even more marked in the

adult. Different parts of the same breast present very different pictures. In pathologic conditions the irregularity may be much more pronounced, but Pallot [3] has said, and I agree with him, that if a breast presents uniform appearances throughout, that breast is not normal. It seems that just as in the ovary only some follicles develop each month, so in the breast only some parts proliferate with each cycle. But one does not know. However, cycle changes have to be allowed for in evaluating lesions of the breast.

The specimen to be described illustrates some of the difficulties encountered in classifying one's material.

Breast 57 was from a woman aged 28, who died after an illness of about two weeks. She had not menstruated since admission to the hospital, ten days before. The endometrium was typical of the estrogenic phase. Each ovary showed numbers of follicular cysts and a degenerating corpus luteum, probably from the last cycle. The result of the Wassermann test was 4 plus. This breast showed extremely well developed lobules in some areas (fig. 6 *A*). Elsewhere the lobules were typically postmenstrual and did not appear to have budded out at all during the cycle. In yet other places budding had occurred, but the ducts ended in small cysts like those of the adolescent breast (fig. 6 *B*). The larger ducts were dilated and contained yellow secretion. If one assumes that death took place toward the end of the estrogenic phase of a menstrual cycle, the findings might be interpreted as follows: The cystic condition of the ovaries (possibly the result of excessive sexual intercourse) would cause increased estrogenic stimulation of the breast—hence the overdeveloped lobules and the adolescent type of ducts. The larger cystic ducts with their contained secretion suggest that a pituitary factor was also involved. The underdeveloped lobules may be those which would have grown during some future cycle or possibly represent the remains of lobules which regressed at some previous date.

Cystic Disease.—If, by study of serial sections, cystic breasts are compared with normal adolescent and adult breasts certain types of cystic proliferation may be distinguished. The first of these I should name "simple cystic disease" or cystic disease of the adolescent type. In this condition a type of growth similar to that occurring in the normal adolescent breast is grafted onto an adult breast which is also responding to the sexual cycle. When viewed three-dimensionally under the dissecting microscope, the cysts closely resemble the cystlike swellings of the growing end branches of the normal developing breast (fig. 7). But unlike these findings in the normal breast, they show enormous variation in size and distribution (fig. 8 *A*). An analysis of the structures seen in a cystic breast of this type leads irresistibly to the conclusion that a stimulus similar to that acting on the breast at puberty is acting on the adult breast which at the same time is under the influence of the cycle.

3. Pallot, G.: Bull. d'histol. appliq. à la physiol. **12**:378, 1935.

The question has arisen and is discussed by Cheatle and Cutler [4]: Are cysts formed by the breaking down of the septums between adjacent ducts? In simple cystic disease this mechanism, if it does occur, is

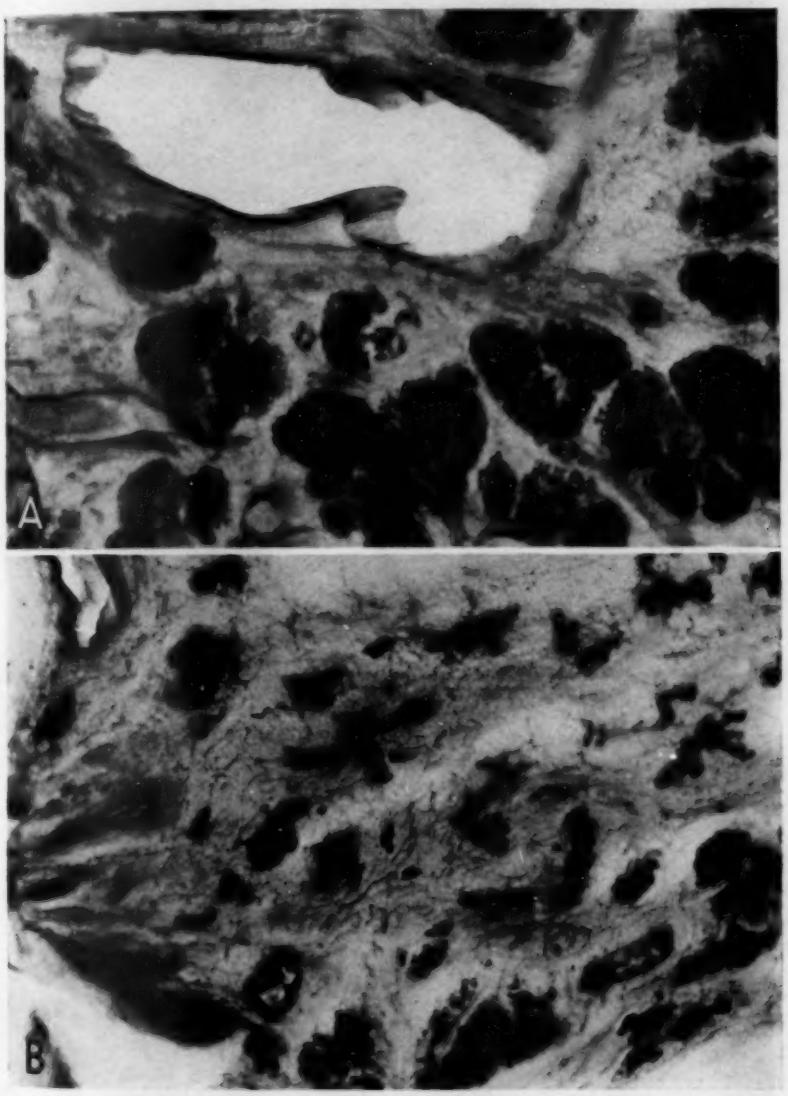

Fig. 6 (breast 57).—Postmortem specimen from a woman aged 28, whose death occurred after two weeks of illness, probably about the end of the estrogenic phase of the cycle. The ovaries were cystic. *A* shows lobules somewhat overdeveloped for the menstrual date and a cystic duct. *B* shows proliferation of the adolescent type (very early simple cystic disease).

4. Cheatle, G. L., and Cutler, M.: Tumours of the Breast, Philadelphia, J. B. Lippincott Company, 1931, p. 102.

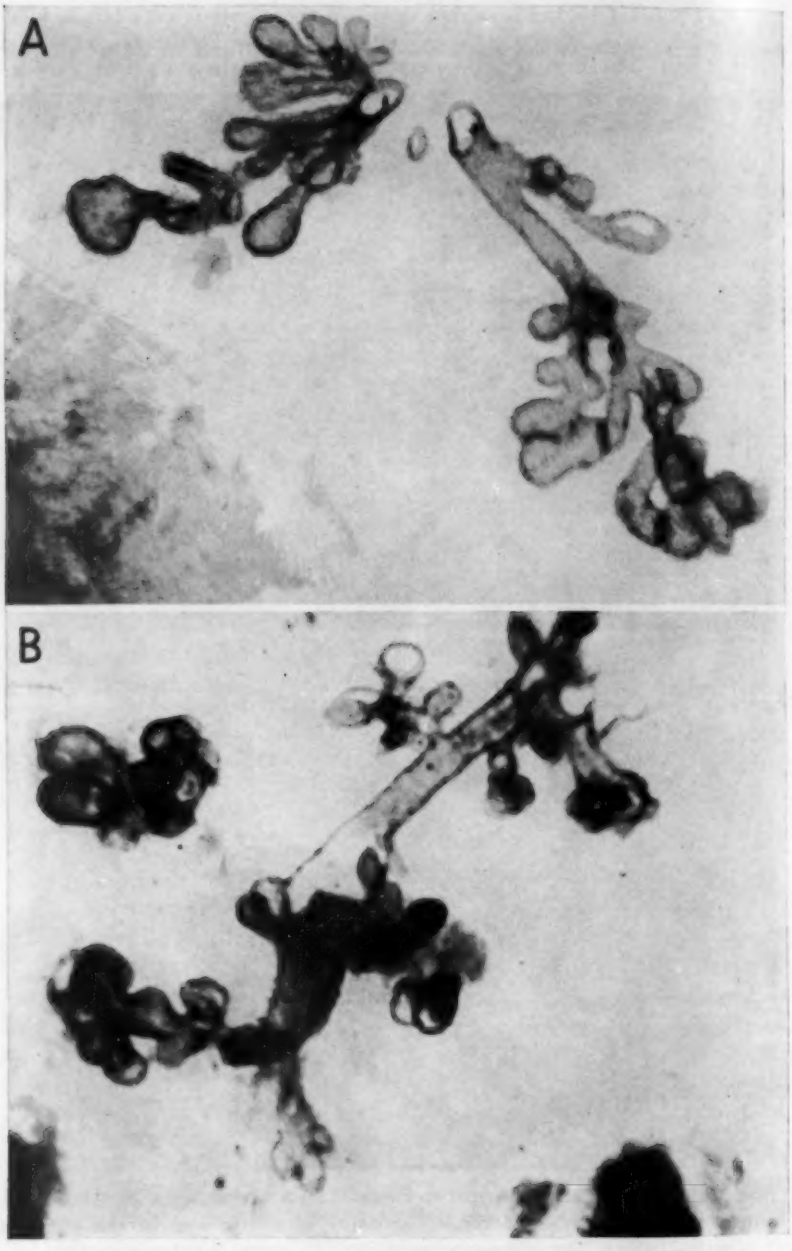

Fig. 7.—*A* (breast 3), terminal portions of two ducts in a breast from a girl 13 years old. The club-shaped and balloon-like terminal ducts resemble those of cystic disease (see *A* in fig. 8). *B* (breast 107), simple cystic disease in the breast of a woman aged 45, who was operated on two days before the menstrual period. Compare with *A* in figure 7.

secondary and relatively unimportant. Only after careful search were one or two clumps of cysts found where septums were wearing very thin and rupture of some of them seemed to have occurred. It must be remembered that ridges similar to those described in the adolescent breast may also be seen here and there in cystic breasts. Often a ridge forming the boundary of the mouth of a cystic branch is hard to distinguish from a ruptured septum.

There is something else that a study of these sections brings out clearly. In one's student days one was careful to differentiate between ducts and lobules. The question has been put: Do cysts arise from ducts or from lobules? Looking at these sections, one cannot help asking what *is* a lobule? In simple cystic disease, cysts form at every stage of branching, presumably because at a given moment the whole duct wall grows instead of only part of it. Studying the sections, I can see no stage of development of breast parenchyma in which this has not happened. The size of the resulting cysts depends in the first instance, of course, on the size of the affected branches. That is why the cysts vary so much. A lobule consists of ducts in their ultimate stage of division. But it is not the static structure that the older school visualized. In simple cystic disease many lobules, if they are lobules, are represented by bunches of cysts (fig. 8 *A*).

The relation of simple cystic disease to fibroadenoma becomes very clear when serial slicer sections of tumors of this type are examined (fig. 8 *B*). Fibroadenoma is of common occurrence in breasts with simple cystic disease and may be considered part of the picture. The tumor consists of an overgrown duct and its branches. The degree of abnormality is apt to vary from branch to branch. Often there is no cystic dilatation, partly because the periductal connective tissue has undergone abnormal proliferation or has failed to regress and partly because the surrounding breast has not yielded to the expansion of the abnormal ducts. Each lobule of the tumor represents the expansion of one unit, that is, one branch with its subdivisions and their periductal connective tissue.

The dependence of the conditions described in the foregoing pages on hormone imbalance is clear not only from their morphologic pattern but from the coexistence in the majority of cases of lesions in related organs, especially in the pelvic organs and thyroid. The necessity for pelvic examination in all cases of disease of the breast has not been sufficiently realized by surgeons. Among 18 cases in which the clinical data are fairly complete there were 16 with evidence of pelvic disease. In 3 there was thyroid disease. Of the 2 subjects who gave no clinical evidence of pelvic or thyroid derangement, the first was married but had never been pregnant, although she did not use contraceptives. The

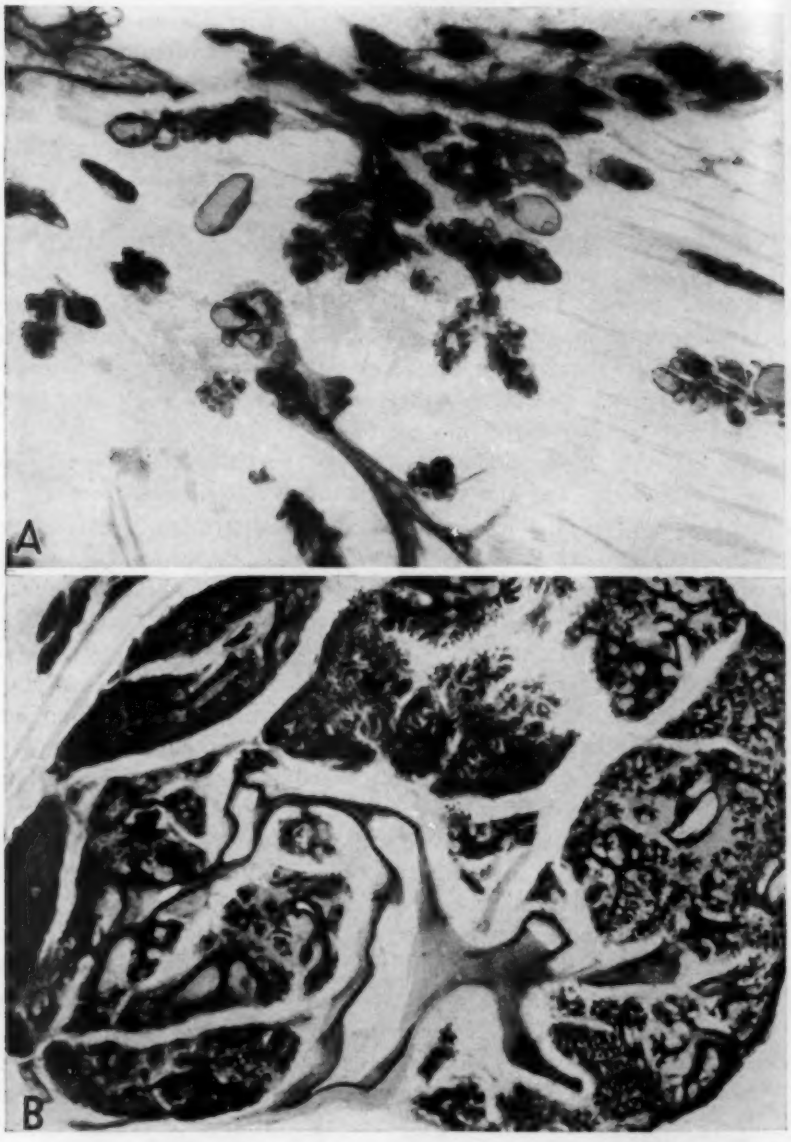

Fig. 8.—*A* (breast 123), simple cystic disease in the breast of a woman aged 38, whose last menstrual period occurred nine days before operation. The bunches of cysts are of all sizes and are intermingled with some more or less normal lobules, but for irregularity in size and distribution the cysts resemble the normal end branches of the developing adolescent breast. *B* (breast 104), fibroadenoma from the breast of a woman aged 42, seven days after the menstrual period. She had early simple cystic disease with fibroadenoma. The tumor consists of a cystic duct and its branches. Each branch shows abnormal proliferation, but the abnormalities are not of the same degree and sometimes not of the same kind; i. e., connective tissue preponderates in some lobules, epithelial structures in others.

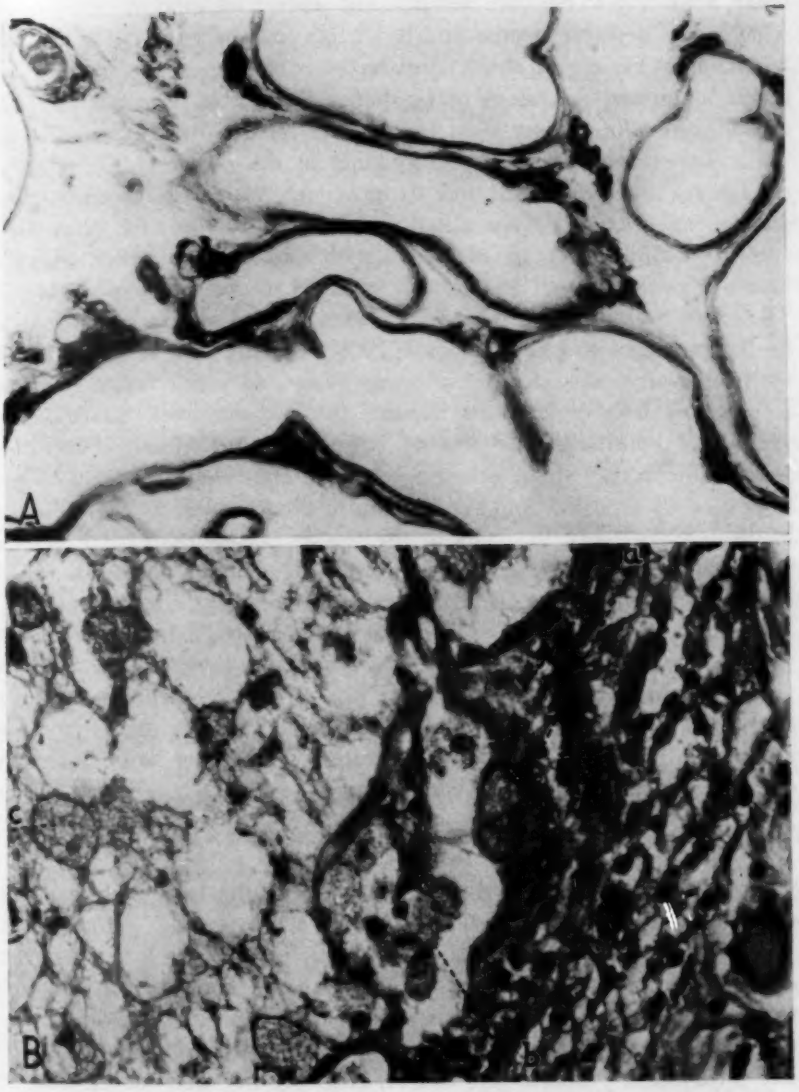

Fig. 9.—*A* (breast 121), secretory type of cystic disease in the breast of a woman aged 49, who had been married twenty-six years, had borne ten children and had no miscarriages and whose menstruation had always been regular. The breast consisted entirely of enormously dilated ducts filled with inspissated secretion. The lobules were completely atrophic. One patch of plasma cell mastitis was present. *B*, early stage of secretory cystic disease in the breast of a woman aged 38. This is a photomicrograph of a part of the wall of a cystic duct (paraffin section; hematoxylin and eosin; high power 4 mm. objective). At *a* are colostrum corpuscles in epithelium lining the wall of the duct; at *b*, colostrum cells in a cavity formed by degeneration of epithelial cells in the center of an area of hyperplasia (see text); at *c*, colostrum cells in the lumen of the duct.

other, according to her own account, had undergone a pelvic examination which gave negative results and had a normal thyroid.

Another variety of cystic disease, allied to the simple cystic type, might be termed "secretory cystic disease." In this form the ducts are dilated, often throughout their length, and filled with inspissated secretion. In extreme examples, every duct in the breast is affected, and the lobules are compressed and atrophic (fig. 9 *A*). Like simple cystic disease, the secretory type also has its origin in distorted physiologic stimuli, possibly those having to do with lactation. In other cases the lesion is more localized and may be confined to one sector of the breast. The cases in which the disease is less advanced also present cysts of the simple type, and one cannot but suppose that just as in the uterus the proliferative precedes the secretory phase, so in the breast secretory is preceded by simple cystic disease. When secretory disease supervenes, the epithelial cells become larger, the protoplasm clearer and colostrum corpuscles make their appearance (fig. 9 *B*). This may occur in heaped-up areas of lining epithelium of dilated ducts as well as in the lobules. Sometimes the cells in the center of the heap show swollen foamy protoplasm. They then degenerate, leaving a space which resembles the lumen of an acinus. The remaining cells, however, also degenerate. The cells lining ducts distended with old inspissated secretion are usually atrophic. One has the impression that once the secretory phase is established degeneration of proliferated epithelial cells in the affected area supervenes sooner or later, and the risk of carcinoma is eliminated. So-called plasma cell mastitis, on the other hand, is directly connected with this lesion as we have tried to show.[5]

SUMMARY

The varieties of cystic disease described in the foregoing pages are the outcome of what might be called a physiologic derangement. Experience has shown that the changes are reversible to a much greater degree than is usually thought. Unfortunately, the simple type is apt to be complicated to a greater or lesser degree by intracystic proliferation, and this, except in its very earliest stages, cannot be compared to any physiologic process. Of course, such proliferation may eventually end in carcinoma, but it is hoped that a better understanding of cystic disease will lead to clearer diagnosis and eventually to prevention of this complication.

5. Rodman, J. S., and Ingleby, H.: Ann. Surg. **109**:921, 1939.

PATHOLOGIC CHANGES FOLLOWING INJECTIONS OF FERRIHEMATE (HEMATIN) IN DOGS

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In the course of a study of the solubility and the titration of hemin and ferrihemic acid, Morrison and Williams [1] prepared a stable solution of disodium ferrihemate [2] of p_H 7.6. We thought such a well buffered solution might be of some value for the relief of anemia, and accordingly some of it was administered intravenously to a dog which had undergone severe hemorrhage, but the result was death of the dog. That intravenous injection of this solution would have a fatal outcome was repeatedly confirmed in apparently normal dogs without previous hemorrhage. Later we became interested in the identification of malarial pigment with ferrihemic acid, and the question was raised whether the toxic symptoms in cases of fulminating malaria might be due to release of ferrihemic acid.

The only previous study of the toxic properties of ferrihemic acid known to us is that of Brown,[3] who concluded that this substance is not an intermediate product in the formation of hemosiderin and bile pigments. The ferrihemic acid used by Brown was markedly toxic, and metabolic disposal of it was difficult. He was convinced of the identity of ferrihemic acid with the pigment of malaria and conceived that the malarial parasite metabolized the protein fraction of hemoglobin, while

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1. Morrison, D. B., and Williams, E. F., Jr.: J. Biol. Chem. **137**:461, 1941.

2. "Hematin" is a general term which has been used for ferriheme and its salts, products formed on the splitting of hemoglobin by acids and alkalis. The more appropriate terms, "ferrihemic acid" and "ferrihemates," as proposed by D. B. Morrison and E. F. Williams Jr. (J. Biol. Chem. **123**:lxxxvii, 1938), will be employed here.

3. Brown, W. H.: J. Exper. Med. **13**:290, 1911; **14**:612, 1911; **15**:579, 1912; **18**:96, 1913; Arch. Int. Med. **12**:315, 1913. Brown, W. H., and Lowenhart, A. S.: J. Exper. Med. **18**:107, 1913.

the ferrihemic acid accumulated as a waste product. Wells [4] noted that the occurrence of ferrihemic acid was reported in cases of congenital hematoporphyrinuria, chromium poisoning, war gas poisoning and acute toxic hemolysis. Michel and Harris [5] reported the presence of ferrihemic acid in the blood plasma and the urine of patients with malaria, as well as of those with pernicious anemia, intravascular hemolysis and sepsis. On the other hand, no toxic effect was observed by Duesberg [6] following the injection of small doses of ferrihemate into human subjects.

Another factor to be considered when dealing with liberation of ferrihemic acid in the blood is the observation of Fairley [7] that free ferrihemic acid added to blood rapidly combines with albumin to form a new product, termed methemalbumin. He suggested that marked intravascular hemolysis might be followed by formation first of ferrihemic acid and later of methemalbumin.

EXPERIMENTAL PROCEDURE AND METHODS

Solutions of disodium ferrihemate, prepared from recrystallized hemin and adjusted to a p_H of 7.6, were injected into dogs intraperitoneally, subcutaneously and intravenously.

By intravenous administration, initial concentrations of 10 to 72 mg. of ferrihemate per hundred cubic centimeters of plasma were obtained. Varying directly with the initial concentration, ferrihemate persisted in the plasma for several hours to two days. Ferrihemic acid was not excreted in the urine. There was occasionally a slight increase in urinary porphyrin, but no elevation of serum bilirubin.

Marked reactions are induced by intravenous injections of ferrihemate; death occurs when an injection is made too rapidly or when excessive amounts are given. In 1 dog anuria developed two days after the injection and persisted for thirty-six hours. Two dogs which survived intravenous administration of ferrihemate were killed on the fourth and sixth days after the injection, respectively.

Intraperitoneal injections were well tolerated. Spontaneous death did not occur in any instance when 200 mg. of ferrihemate was given thrice weekly for periods ranging to nineteen weeks.

Three dogs were used for subcutaneous injections. During a period of eight to twelve days each dog received five injections of 20 mg. of ferrihemate. Injected areas became so edematous and tender that further injections were not attempted.

All autopsies were made within a few hours after death. Animals were killed usually by ether, pneumothorax or bleeding. Tissues were fixed in solution of formaldehyde U. S. P. Paraffin sections and hematoxylin-eosin stains were used routinely. Certain sections were studied by the berlin blue and Gomori stains and by microincineration for iron, and by the azocarmine, Masson [8] trichrome, Mallory connective tissue and Verhoeff-Van Gieson stains.

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4. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.
5. Michel, H. O., and Harris, J. S.: J. Lab. & Clin. Med. **25**:445, 1940.
6. Duesberg, R.: Arch. f. exper. Path. u. Pharmakol. **174**:305, 1933.
7. Fairley, N. H.: Brit. M. J. **2**:213, 1940.
8. Masson, P.: J. Tech. Methods **12**:75, 1929.

RESULTS

Pigment accumulations, vascular abnormalities and renal lesions comprised the pathologic findings. Dosage of ferrihemate, route of administration and duration of effect were factors which influenced the results.[9] The accumulation of pigment occurred most consistently, whereas the occurrence of vascular and renal changes was quite variable and independent of dosage or intervals of time. The acute renal lesions and particularly the more severe degenerative changes in the kidneys followed intravenous injections. Intraperitoneal injections usually had only local effects or produced milder and chronic changes.

Pigment Accumulations.—In all dogs, generalized pigmentation was exhibited throughout the reticuloendothelial system. At the site of subcutaneous injection there was local deposition of brownish black pigment, the stained area extending for a distance of several centimeters. This gross discoloration was still very prominent after ten days. In section, the pigment was found to be concentrated in the cytoplasm of large mononuclear phagocytic cells and occasional giant cells. A mild inflammatory process was evident, with slight vascular congestion and a few polymorphonuclear leukocytes. The prussian blue reaction for inorganic iron was negative, although iron could be demonstrated by microincineration. Apparently the pigment is very stable and is only slowly transported to accumulate in phagocytic cells of lymph nodes, spleen, liver, sinusoids and bone marrow. After a period of storage in such locations, the pigment gives a positive microchemical reaction for iron with Gomori's method. It is uncertain, however, whether this result reflects a slow metabolism of the ferrihemate or the presence of hemosiderin from hemorrhage.

After intraperitoneal injection of ferrihemate, localized accumulation of pigment was very pronounced at least as late as seven days after the last injection, which was the longest interval before autopsy. The entire visceral and parietal peritoneum was stained deep brownish black, though the surfaces retained their normal sheen. Fluid in the peritoneal cavity was not excessive, and gross evidence of inflammatory reaction was minimal. The mesentery and the omentum were considerably thickened and markedly discolored. The pigment was held in large numbers of mononuclear phagocytic cells; these were most numerous in the thickened omentum, where there was occasionally a mild inflammatory reaction (fig. 1 *B*). Local lymph nodes and reticuloendothelial tissue contained some pigment. It is evident that the pigment can be held locally in very large amounts and is released only very slowly, via the lymphatics, to the

9. Morrison, D. B.; Williams, E. F., Jr., and Anderson, W. A. D.: To be published.

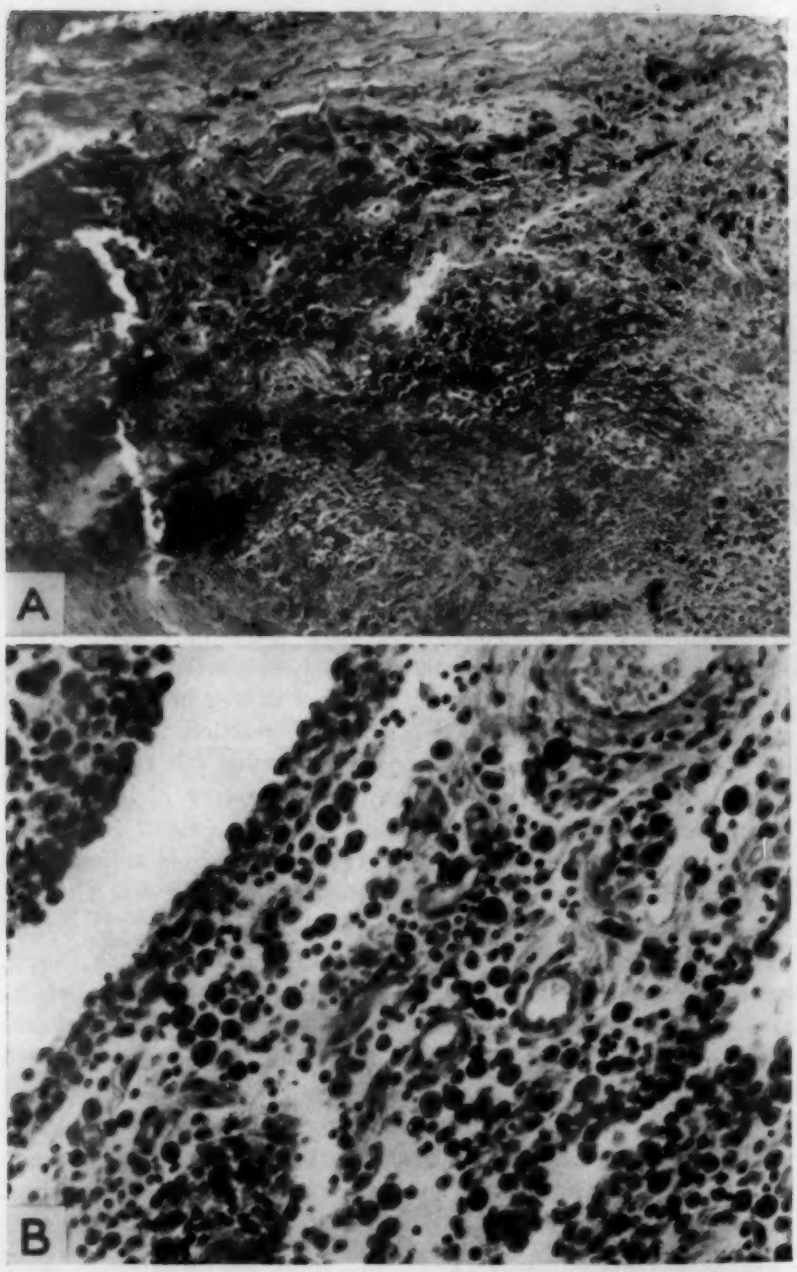

Fig. 1.—*A*, spleen showing pigment deposits, fibrosis and pigmentation of fibrils; a siderofibrotic nodule; × 150. *B*, mesentery showing the accumulation of many large mononuclear cells containing pigment; × 300.

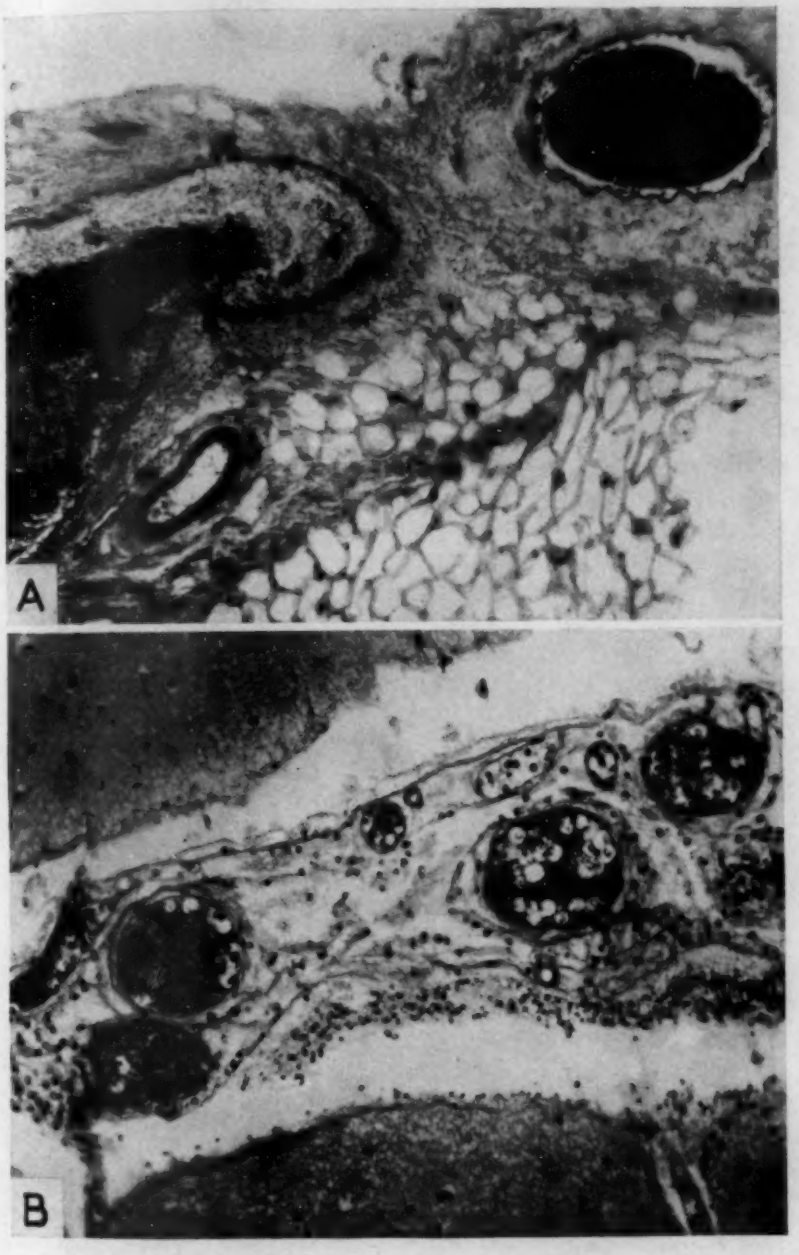

Fig. 2.—*A*, masses of pigment and thrombosis in omental vessels; × 200. *B*, thrombosis and pigment in meningeal vessels; × 200.

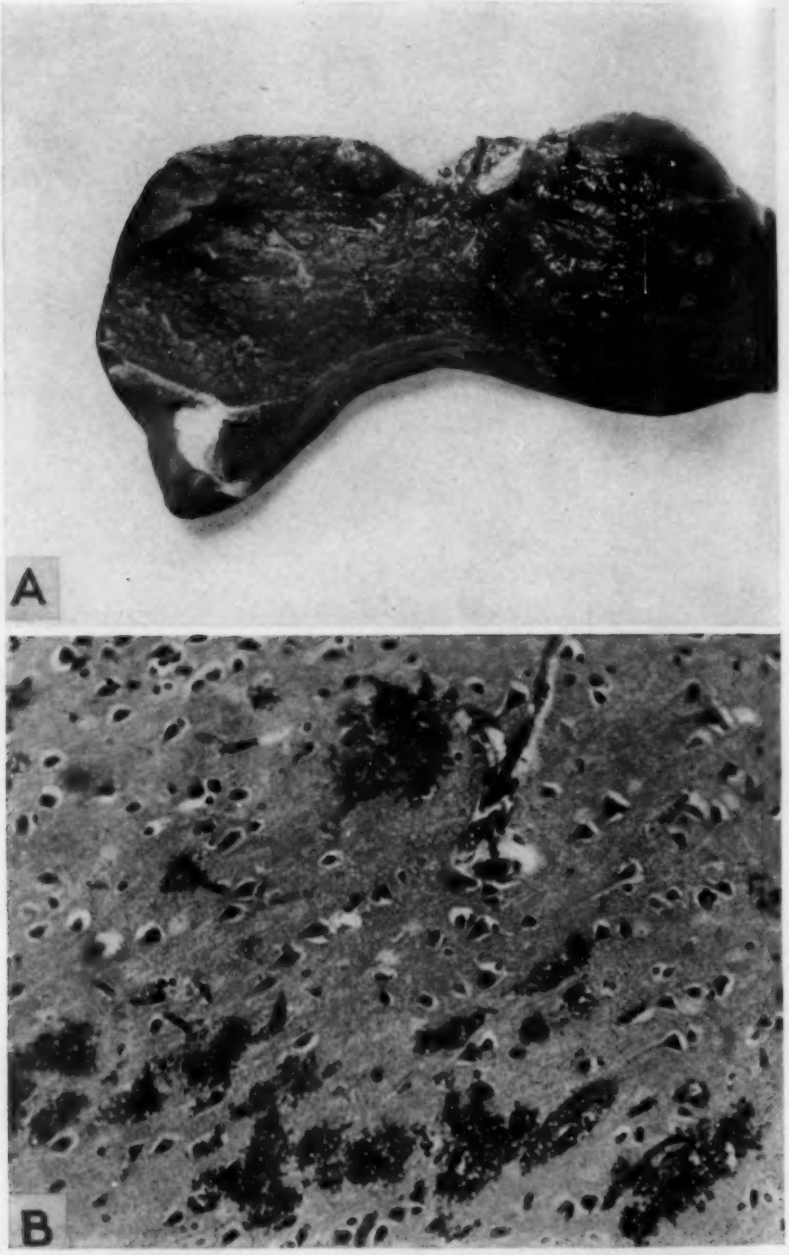

Fig. 3.—*A*, portion of spleen showing a localized area of massive hemorrhage. *B*, multiple small hemorrhages in cerebral tissue; × 200.

storehouse of the reticuloendothelial system, in which, eventually, enormous amounts of the pigment accumulate.

Large local capacity for storage with but slow release probably accounts for tolerance of large doses of ferrihemate given repeatedly by the intraperitoneal route. Quantities of ferrihemate ranging from 1,400 mg. in forty-eight days to 3,200 mg. in thirty-eight days, and even larger doses over longer periods, were given intraperitoneally with little exhibition of toxicity.

Storage of pigment in the spleen occasionally produced siderofibrotic nodules (Gandy-Gamna bodies) entirely similar to those found in sickle cell anemia [10] or Banti's syndrome (fig. 1 *A*). Otherwise, splenectomy prior to a series of intraperitoneal injections was without effect on toxicity or lesions.

Vascular Abnormalities.—In contrast with the relatively mild vascular effects produced by intraperitoneal injection of ferrihemate is the marked effect seen in the vascular system after intravenous and, to a lesser degree, after subcutaneous administration. Generalized vasodilatation, hemorrhages varying in size from microscopic extravasations to massive hematomas, thromboses and sometimes infarction occur. Many of the thrombi contained masses of pigment, but a thrombus of the ordinary fibrin type was also a frequent finding (fig. 2). Vascular congestion and petechial hemorrhages were common in the subarachnoid space and the central nervous system, and frequently would explain the toxic effects and convulsions which often followed intravenous injection (fig. 3 *B*). Subendocardial and myocardial hemorrhages were also frequent. In 1 case a massive hematoma was formed in the spleen (fig. 3 *A*), and in 1 instance there was a large subcutaneous hemorrhage. Submucosal hemorrhages of the bladder and the intestine or extravasations beneath the capsule of the liver were common occurrences. Thrombotic masses, of the usual type or composed mainly of pigment, were found in various regions, including pulmonary, meningeal and intrarenal blood vessels.

Renal Lesions.—Renal changes of some kind were demonstrated in most of the experiments, although the lesions were inconstant in severity and form.

With intraperitoneal administration, glomerular lesions of a chronic nature were produced, but these were not extensive enough to cause renal failure during the period of observation. In one experiment, after intraperitoneal injections had been given (with rest intervals) for more than eight months, some pigment was found within the epithelial cells of the renal convoluted tubules; this pigment did not have the appearance or give the reactions of hemosiderin.

10. Diggs, L. W.: J. A. M. A. **104**:538, 1935.

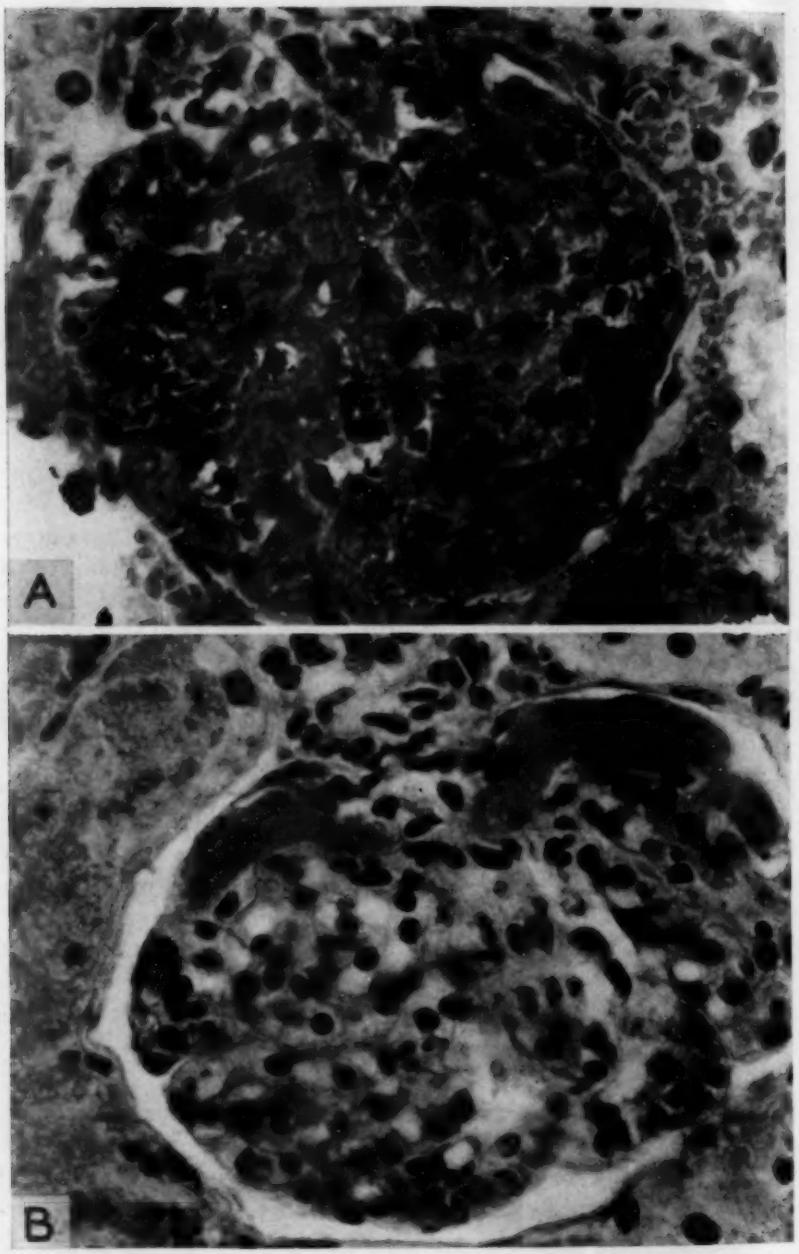

Fig. 4.—*A*, acute congestion in a glomerulus, all capillaries fully distended; × 400. *B*, early hyaline changes in a glomerulus and tubular degeneration; × 400.

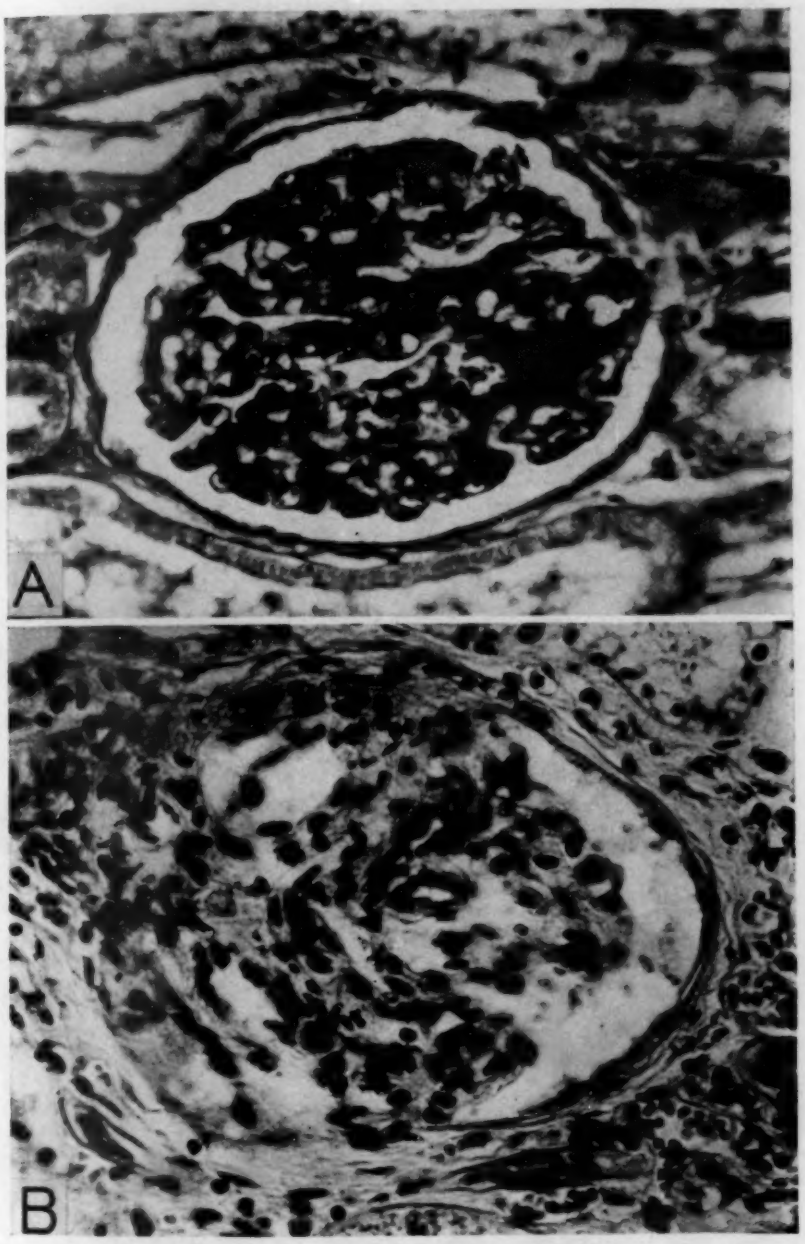

Fig. 5.—*A*, marked thickening of a glomerular basement membrane; azocarmine stain; × 350. *B*, partial hyalinization and capsular adhesions of a glomerular tuft; × 350.

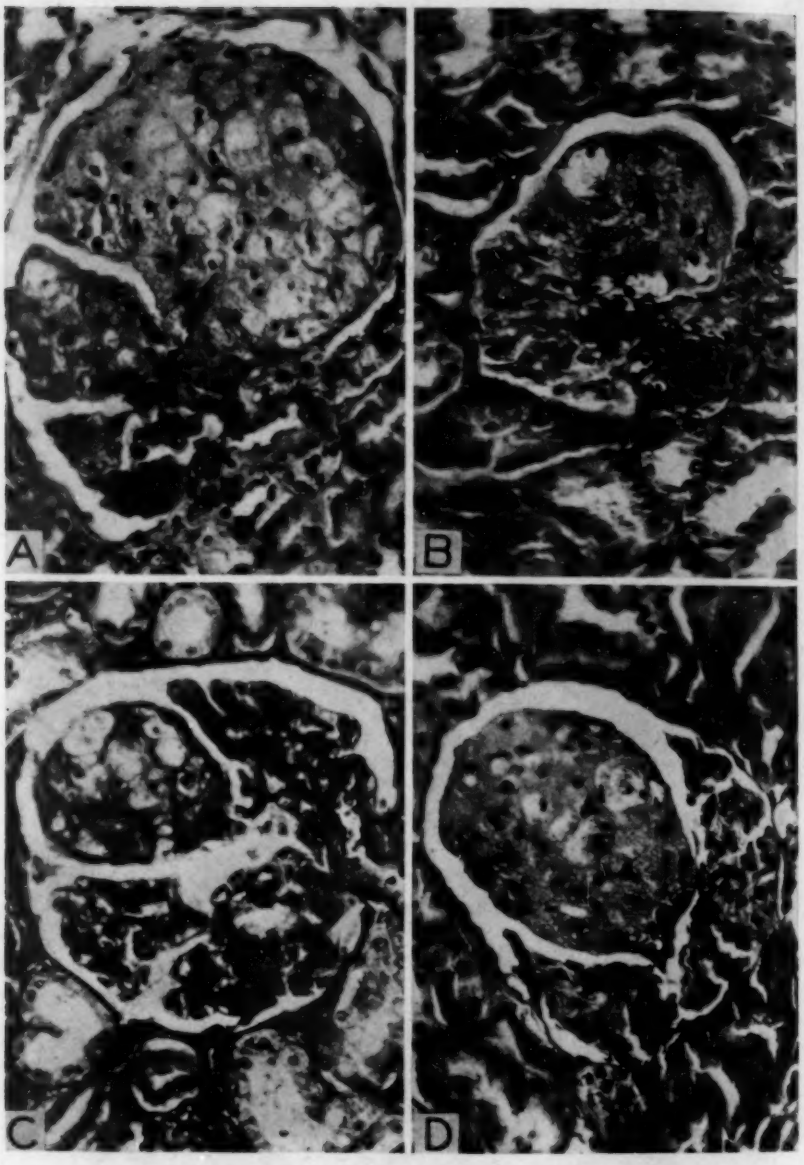

Fig. 6.—*A*, isolated lobules of glomerular tuft with marked dilatation of vessels and beginning hyalinization; × 250. *B* and *C*, other stages of the process, showing widely dilated vessels in one lobule of a tuft, but with more advanced hyalinization. *B*, × 250; *C*, × 2,500. *D*, lobule of a glomerular tuft almost completely hyalinized; × 250.

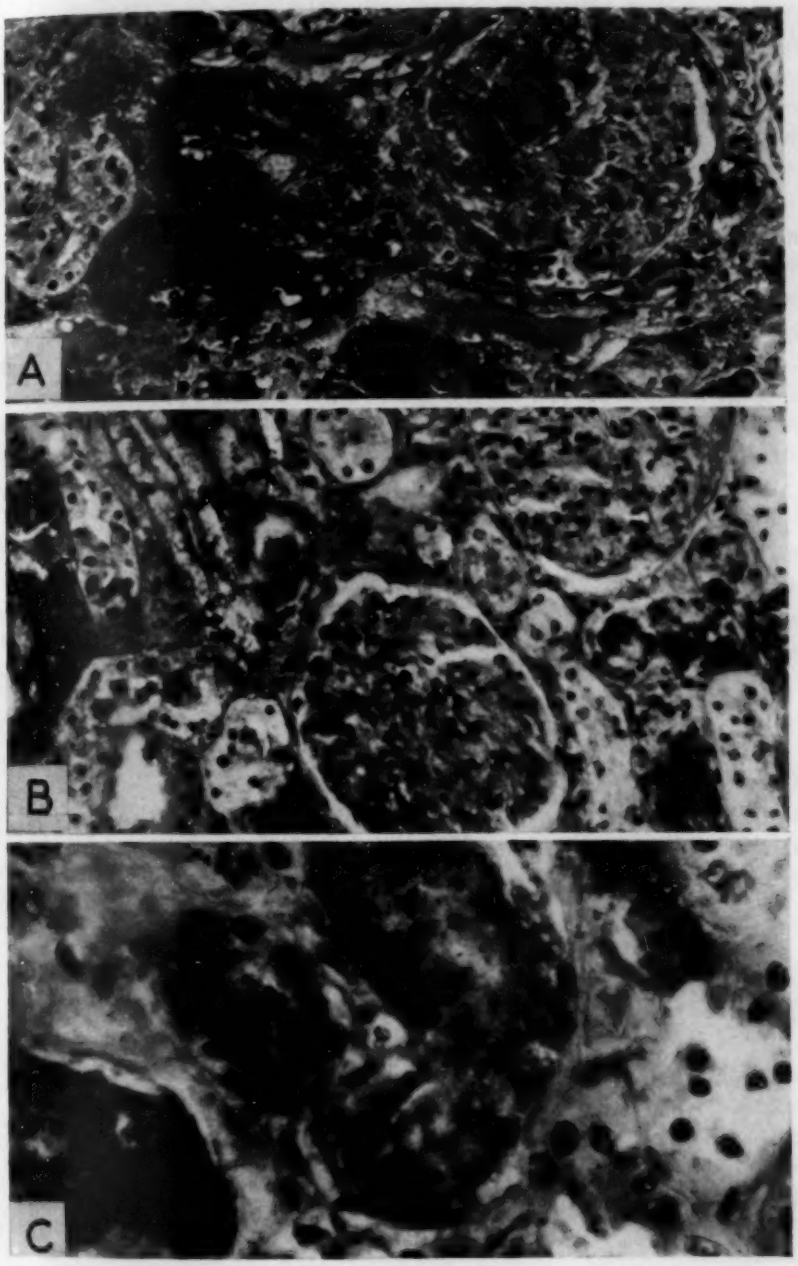

Fig. 7.—Acute renal damage following intravenous injections of hematin: *A*, degeneration and necrosis of convoluted tubular epithelium, with some calcification; × 300. Some tubules contain hyaline casts. Hyaline masses are seen in a glomerular tuft. *B* and *C*, severe tubular degeneration, with dark granular hematoxylin-staining masses in necrotic tubules. *B*, × 300; *C*, × 400.

Severe renal damage occurred in these experiments usually only after intravenous injections of ferrihemate. The kidneys shared in the general vascular injury. During early intervals after injection many glomeruli were markedly engorged (fig. 4 *A*). Glomerular tufts showed hyaline thrombi (fig. 4 *C*), slight cellular proliferation and, occasionally, some leukocytic infiltration. Later changes in glomeruli consisted of capsular adhesions, thickening of basement membranes and hyalinization to varying degrees (fig. 5). Frequently hyalinization was limited to one portion of a glomerulus. The process began as lobulation of the glomerular tuft and marked dilatation of the capillaries of one lobule. The lumens remained widely dilated for a long period, but the walls gradually underwent hyaline thickening, and the cells disappeared. Finally this portion of the glomerulus became converted into a hyaline mass (fig. 6). Ayer [11] described somewhat similar glomerular changes in association with deep jaundice.

Changes in the cortical tubules were more constant than glomerular lesions. Degeneration of convoluted tubules varied from mild granularity and swelling to massive necrosis with disintegration of cells, early calcification and accumulation of hematoxylin-staining granules and masses. Casts occurred in the kidneys with severe tubular injury, but pigment casts by themselves were never a prominent feature (fig. 7).

COMMENT

Storage of large amounts of ferrihemate pigment in phagocytic cells of the reticuloendothelial system, where the pigment remains, relatively innocuous and probably unaltered, for long periods, suggests that ferrihemate is not a normal intermediate product of the metabolism of bile pigment. This suggestion is supported by the failure of plasma bilirubin to increase significantly after the administration of ferrihemate.[12]

While ferrihemate may thus remain apparently indefinitely in the tissues, stored in phagocytic cells, as an almost inert and harmless substance, its presence in the blood stream causes severe damage. The injury is largely related to vascular reactions, which include dilatation and congestion of small vessels, hemorrhages and thromboses. One may speculate whether ferrihemate per se, or some compound of ferrihemic acid and blood proteins, such as Fairley's [7] methemalbumin, is the responsible agent. Most of the observed lesions can be accounted for by the multiple small thrombi, without invoking any intrinsic toxicity of ferrihemate.

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11. Ayer, D.: Arch. Path. **30**:26, 1940.

12. Morrison, D. B.; Williams, E. F., Jr., and Anderson, W. A. D.: J. Biol. Chem. **133**:lxx, 1940.

The production of acute renal lesions by intravenous injections of ferrihemate is of particular interest for its bearing on the problem of renal changes induced by intravascular hemolysis. The establishment of blood banks and the increasing use of transfusions have made the question of toxicity of hemolyzed blood one of practical importance. The impression has prevailed that hemolyzed blood is toxic. Intravascular hemolysis has been thought to be the chief cause of reactions and deaths from injections of incompatible blood. Similar effects, particularly in the kidneys, are seen in other examples of massive intravascular hemolysis, as in blackwater fever.

The mechanism of renal damage in such circumstances is not clear. Renal tubular obstruction by precipitated pigment has been shown to occur when the urine is acid.[13] It has been difficult, however, to believe that mechanical blockage alone could account for all of the loss of renal function observed. Other toxic factors have been suggested by Bordley [14] and Kimmelstiel.[15] Marked tubular degenerative lesions were described by Goldring and Graef.[16] DeGowin, Warner and Randall [17] expressed the belief that although two separate processes operate, namely, obstruction of tubules by pigment and a nephrotoxic process causing tubular degeneration and necrosis, the nephrotoxic process independently could cause renal insufficiency. They concluded that renal insufficiency following hemolysis is caused in the majority of instances by a nephrotoxic substance which causes degeneration of tubular epithelium and interstitial edema.

The inconstancy of the nephrotoxic action, its independence of quantity of blood transfused and the fact that injections of hemoglobin have no obvious deleterious effects in many instances suggest that the toxic factor is some abnormal breakdown product or derivative of hemoglobin. Fairley [7] suggested that when intravascular hemolysis is extensive, the breakdown of hemoglobin may proceed not only by way of the formation of hemosiderin and bile pigment but also by a pathway which leads to formation of ferrihemic acid. It is worthy of note that the renal lesions which developed in our dogs following intravenous injection of ferrihemate were similar to the nephrotoxic changes described by Bordley,[14] Goldring and Graef [16] and DeGowin, Warner and Randall [17] in cases of transfusion reactions. Our observations suggest that such

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14. Bordley, J.: Arch. Int. Med. **47**:288, 1931.

15. Kimmelstiel, P.: Am. J. Path. **14**:737, 1938.

16. Goldring, W., and Graef, I.: Arch. Int. Med. **58**:825, 1936.

17. DeGowin, E. L.; Warner, E. D., and Randall, W. L.: Arch. Int. Med. **61**:609, 1938.

lesions are secondary to vascular and circulatory changes rather than due to any direct action of a nephrotoxin on the tubular cells.

Recalling that ferrihemate is the pigment of the malarial parasite, we may note here the similarity of the lesions found in malignant tertian malaria to the vascular reactions, particularly the hemorrhages and thromboses, observed in the animals of our experiments. Brown [3] also has commented on these similarities. The actual renal lesions in our animals are more like those found in blackwater fever with marked nephrotic changes. With ferrihemate, however, intrarenal obstruction by pigment casts was never an important feature, as it may be in blackwater fever and transfusion reactions.

The more chronic glomerular lesions, while indicating severe and permanent damage to some nephrons, were less widespread and more inconstant than the tubular lesions. In no instance did the glomerular changes appear sufficient to cause renal failure. The glomerular changes appear to be explicable on the basis of vascular injury and obstruction in the tufts.

SUMMARY

Ferrihemate when injected into dogs is capable of producing acute and chronic changes in the kidneys, the reticuloendothelial system and the vascular system.

When stored in the reticuloendothelial system, ferrihemate remains as an inert and relatively harmless substance, which the body appears to have little ability to metabolize.

Marked vascular reactions followed injection, particularly intravenous injection, of ferrihemate. The vascular changes consisted of dilatation and congestion of small vessels, hemorrhages and thromboses. Small hemorrhages were common in the central nervous system.

The renal lesions were similar to those which may follow marked intravascular hemolysis, as from transfusion of incompatible blood, but without as marked tubular blockage. The renal degenerative changes were probably due to vascular injury and obstruction rather than to any inherent specific toxicity of ferrihemate.

Many of the changes following injections of ferrihemate are similar to lesions which occur in malaria and blackwater fever.

Siderofibrotic nodules of the spleen (Gandy-Gamna bodies) resulted from deposition of ferrihemate in the spleen.

COMPOSITION OF THE LIVER

ITS UNIFORMITY WITH RESPECT TO THE CONCENTRATION OF CERTAIN BIOCHEMICAL CONSTITUENTS IN DIFFERENT PARTS OF THE SAME LIVER

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AND
J. D. STEWART, M.D.
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A specimen of human liver removed for biopsy during a surgical operation is necessarily small and comes from the more accessible part of the organ. When chemical analyses are performed on such a specimen, the significance of the data obtained depends on the degree to which the tissue studied can be taken as representative of the whole. We have for some time made biochemical studies on hepatic tissue removed at operation under varying conditions, and proper interpretation of the results required some knowledge of whether fair samples were obtained by our technic.

A search of the literature shows that most workers have made chemical analyses of postmortem liver tissue after grinding up the entire liver or large portions of it,[1] thus avoiding the question of uniformity of composition. In one study samples were taken from the livers of dogs under anesthesia for chemical analysis, and the statement is made that each sample was large and that the periphery of the liver was avoided.[2]

A divergence of opinion as to the uniformity of distribution of vitamin A in the liver is noticeable. Lindqvist stated that he found a standard deviation of 3.78 in concentrations of vitamin A in 22 specimens of the same lobe of the human liver, but a 75 per cent difference between lobes.[3] Sen and Sharma[4] stated that there may be a 10 per cent variation between lobes, while Moore[5] found the concentration of vitamin A the same throughout the organ.

From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital.

1. (*a*) Halliday, N.: J. Lab. & Clin. Med. **25**:926, 1940. (*b*) Outhouse, E. L., and Forbes, J. C.: ibid. **25**:1157, 1940.
2. Rubin, S. H.; Present, C. H., and Balli, E. P.: J. Biol. Chem. **121**:19, 1937.
3. Lindqvist, T.: Acta med. Scandinav., 1938, supp. 97, p. 1.
4. Sen, K., and Sharma: Indian J. Vet. Sc. **6**:128, 1936.
5. Moore, T.: Biochem. J. **1**:331, 1937.

TABLE 1.—*Values Obtained from Eleven Patients*

Patient's Age, Yr.	Diagnosis	Hours Post Mortem	Weight of Liver, Gm.	Sample	H_2O Content, Per Cent	Vitamin A, Units per Gm.	Fatty Acids, Gm. per 100 Gm.	Cholesterol	
								Total, Gm. per 100 Gm.	Ester, Gm. per 100 Gm.
62	Rectal cancer, malnutrition	3	.....	1	70.0	27	9.2	0.51	0.22
				2	69.7	114	10.2	0.41	0.26
				3	69.5	60	10.8	0.45	0.21
				4	72.8	36	7.0	0.53	0.28
				5	74.5	43	5.9	0.52	0.21
78	Cerebral hemorrhage, diabetes mellitus	28	1,825	1	74.2	300	5.1	0.44	0.14
				2	72.3	300	5.5	0.36	0.18
				3	72.3	230	5.4	0.41	0.16
				4	74.8	200	3.6	0.33	0.12
				5	75.0	250	4.5	0.34	0.14
50	Lymphoblastoma	26	1,900	1	76.9	108	3.1	0.39	0.19
				2	76.6	154	3.0	0.41	0.16
				3	76.6	129	2.4	0.40	0.21
				4	76.1	54	2.4	0.42	
				5	76.9	47	2.1	0.38	0.18
58	Mesenteric thrombosis	10	1,100	1	75.0	833	2.3	0.52	0.46
				2	75.9	1,282	2.8	0.50	0.31
				3	75.5	910	2.9	0.60	0.60
				4	74.6	428	2.6	0.50	0.46
				5	75.8	430	2.7	0.59	0.51
62	Tumor of brain	18	1,916	1	74.7	35	4.4	0.18	0.16
				2	73.7	17	5.6	0.19	0.17
				3	73.1	17	5.8	0.17	0.16
				4	78.3	13	2.5	0.08	0.08
				5	76.6	7	2.5	0.10	0.09
65	Cirrhosis, diabetes mellitus, pyelonephritis	8	1,575	1	78.0	120	1.8	0.22	0.12
				2	78.3	60	1.9	0.26	0.11
				3	78.6	120	1.7	0.34	0.08
				4	79.3	30	1.5	0.25	0.08
				5	78.4	30	1.5	0.24	0.11
64	Pneumonia, uremia	19	1,700	1	78.4	160	1.5	0.28	0.10
				2	77.3	160	1.7	0.38	0.10
				3	77.8	160	1.6	0.37	0.11
				4	76.5	80	1.6	0.42	0.11
				5	78.2	160	1.4	0.40	0.11
48	Cirrhosis, chronic alcoholism	3	4,000	1	67.8	91	15.4	0.35	0.23
				2	69.6	91	14.5	0.33	0.17
				3	68.8	91	13.8	0.34	0.18
				4	69.2	91	14.5	0.34	0.19
				5	69.2	67	13.7	0.32	0.24
45	Hypertensive heart disease	5	1,810	1	76.6	603	3.4	0.22	0.19
				2	77.5	903	2.8	0.20	
				3	77.3	903	3.2	0.22	0.10
				4	76.7	603	3.3	0.23	0.14
				5	77.7	306	3.0	0.17	0.09
45	Tumor of brain	8	1,200	1	75.3	60	3.5	0.20	0.17
				2	75.5	60	3.2	0.20	0.18
				3	75.0	66	3.5	0.24	0.16
				4	75.9	60	3.3	0.20	0.17
				5	76.7	30	2.3	0.17	0.15
57	Cerebral hemorrhage, diabetes mellitus	4	1,650	1	74.1	140	5.2	0.28	0.16
				2	73.6	280	5.4	0.31	0.13
				3	72.7	240	5.3	0.30	0.19
				4	72.3	140	5.7	0.31	0.19
				5	73.3	140	5.2	0.26	0.15

METHODS

Hepatic tissue from 12 patients who died of various diseases was studied. Samples comparable in size to those safely removable at operation, i. e., 1 to 2 Gm., were taken and numbered from the following sites:

1. Anterior margin of the right lobe.
2. Posterior margin of the right lobe.
3. Center of the right lobe.

TABLE 2.—*Analyses of Twelve Samples from the Same Liver* *

Sample	H_2O Content, Per Cent	Vitamin A, Units per Gm.	Fatty Acids, Gm. per 100 Gm.	Cholesterol	
				Total, Gm. per 100 Gm.	Ester, Gm. per 100 Gm.
1	79.3	167	0.8	0.26	0.06
2	79.0	84	0.7	0.26	0.05
3	78.2	133	0.8	0.20	0.06
4	79.6	100	0.8	0.33	0.07
5	80.2	117	0.8	0.28	0.05
6	79.7	167	0.7	0.33	0.08
7	79.9	100	0.7	0.31	0.09
8	79.4	133	0.8	0.26	0.05
9	79.6	167	0.7	0.29	0.06
10	79.6	167	0.8	0.24	0.07
11	79.1	100	0.7	0.27	0.06
12	78.2	167	0.8	0.29	0.05
S. D.	0.54	27.0	0.055	0.037	0.013

* The analyses were made at autopsy in a case of chronic ulcerative colitis, intestinal obstruction and peritonitis. Samples were taken from various parts of the liver four hours after death. The patient had received a diet high in protein and high in vitamins. The standard deviations were computed (S. D.).

TABLE 3.—*Multiple Determinations of Vitamin A Concentration in the Same Liver Sample* *

Portion	Vitamin A, Units per Gm.
1	148
2	138
3	100
4	134
5	100
6	108

* The analyses were made on portions of a liver sample weighing 4.0 Gm.

4. Anterior margin of the left lobe.
5. Posterior margin of the left lobe.

The interval after death varied in different cases, but the samples were subjected to analysis immediately after removal. The hepatic capsule when visible on a specimen was not removed. Approximately 0.6 Gm. of tissue was wiped quickly on a piece of filter paper and weighed in a tared weighing bottle for studies of moisture and fat. The weighing bottle was placed in a drying oven at 110 C. for forty-eight hours, reweighed and the water content calculated. The dry liver tissue was then pulverized in a mortar, reweighed and placed in a 100 cc. volumetric flask with about 75 cc. of a 3 to 1 alcohol-ethyl ether mixture; this mixture was allowed to reflux on a water bath for twenty-four hours. When cooled, the volume

was made up to 100 cc. with the alcohol-ether mixture, and the whole was then filtered through fat-free filter paper. A 65 cc. aliquot was used for a determination of the total fatty acid content by the Stoddard and Drury[6] method; 20 cc. was used for a determination of the cholesterol esters and 10 cc. for a determination of the total cholesterol,[7] the only departure from the Bloor method being in the use of a 20 to 1 acetic anhydride-sulfuric acid mixture as suggested by Outhouse and Forbes.[1b] A sample of approximately 0.2 Gm. was weighed and placed in 5 per cent potassium hydroxide for determinations of vitamin A as described by Davies,[8] final readings being made against known standards in a comparator block. All values are expressed as percentages of wet weights except those for vitamin A, which are given in U. S. P. units per gram of whole liver tissue. Analyses for glycogen were not done since tissues obtained at postmortem examinations rather than fresh tissues were under study.

COMMENT

The values obtained in 11 cases are shown in table 1. Table 2 gives the analyses of 12 samples from various parts of the same liver, samples 1 to 5 being taken at the usual sites. Table 3 gives values for vitamin A concentration, obtained by doing multiple analyses on the same sample of liver tissue, and illustrates the fact that determinations of this type are not of a high order of accuracy.

A rather high degree of uniformity appears to prevail throughout the liver with respect to all the factors analyzed except vitamin A, and the latter substance shows no regular variation in different parts of the liver. The irregularity in the concentration of vitamin A is due in part at least to the fact that the analytic method used has an inaccuracy of 20 per cent, according to our findings and those of Davies.[8] A study of vitamin A concentration in the liver on samples from the present autopsy material and in 38 surgical cases has shown a range of variation from 0 to 1,500 units per gram of tissue; hence the analytic method permits a satisfactory appraisement of hepatic vitamin A storage.

SUMMARY

Different parts of the liver show at autopsy a fairly high uniformity of composition with respect to water content, fatty acids and free and total cholesterol. The distribution of vitamin A is more uneven, but this finding may be due largely to the inadequacy of the analytic method

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8. Davies, A. W.: Biochem. J. **27**:1770, 1933.

SCLEROTIC AREAS IN SKULLS AFFECTED WITH PAGET'S DISEASE

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The investigation of bone changes in skulls affected by Paget's disease resulted in a report concerning conditions in the temporomandibular joint.[1] The present report deals with a histologic study of the sclerotic areas in such skulls.

It is known that the roentgenographic appearance of bone involved in Paget's disease is blurred; dense and porous areas alternate in a blotchy way. The microscopic picture of the involved bone has been described in detail by several investigators. This article deals solely with the sclerotic areas in the skulls, exclusive of all other features, such as inflammatory reactions, healing and recurring processes and pericranial and dural growth of the cranium. The literature makes reference to sclerotic areas in skulls involved in Paget's disease without investigating details. The researches of Schmorl,[2] Freund,[3] Pines[4] and Erdheim[5] are of particular value in this connection.

The investigated material consisted of four complete skulls from the collection of the late J. Erdheim, of Vienna. The sclerotic areas in these four skulls were identical and therefore only one skull will be described in detail.

This skull was that of a 65 year old man. It showed many sclerotic areas. The cranium varied in thickness from 1 to 3 cm. The maximum thickness occurred in the frontal region. At autopsy the cranium was divided into two parts in a sagittal direction; these two parts were divided into three parts in a frontal direction. In this way, two frontal, two parietal and two occipital pieces of the cranial bone were obtained. Roentgenograms of these six pieces were made. Figure 1 *A* shows a roentgenogram of a parietal segment. Each of the six segments of the cranium was cut into five or six disks, as shown by the lines in figure 1 *A*. Thus, forty-five disks were obtained from the segments, and each was roentgenographed in a frontal direction. A roentgenogram of one of these disks is shown in figure 1 *B*.

From the Foundation for Dental Research of the Chicago College of Dental Surgery, the Dental Department of Loyola University.

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3. Freund, E.: Virchows Arch. f. path. Anat. **274**:1, 1930.
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The roentgenograms of the six segments and the forty-five disks disclosed all the sclerotic, as well as the porotic, areas in the calvaria. The forty-five disks were cut into small pieces, according to the lines shown in figure 1 *B*. These pieces were decalcified and sectioned for microscopic study. The skull was divided into two hundred and fifty-four blocks, and the sections from these blocks were used for the present study.

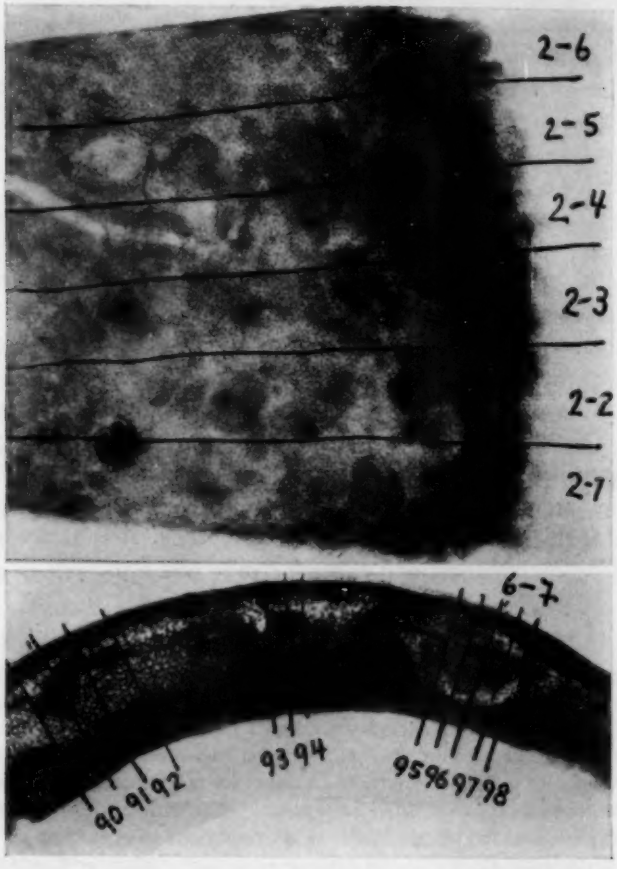

Fig. 1.—*A*, parietal segment of a skull involved in Paget's disease, showing sclerotic and porotic areas. The lines indicate the direction in which this bone was cut into six disks. *B*, roentgenogram of one of the forty-five disks obtained from the involved skull. The ink lines indicate the blocks into which the disk was sectioned and studied microscopically.

On observing this material, it became evident that the sclerotic areas in the affected skull were not uniform in their structure, origin or position within the bone. Six structurally distinct types of sclerotic areas were observed.

Figure 2 *A* shows the general view of a section through the layers of the cranial bone from the pericranium (*a*) to the dura mater (*b*). Between two layers showing active Paget's disease (*c* and *d*), areas can be observed where the process has stopped (*e* and *f*), at least temporarily. Figure 3 *A* shows part of a sclerotic area in higher magnification. This sclerotic area is designated as type 1. It consists of compact, eburnized bone which is perforated by nutritional channels, most of which are very narrow, while some are wider. There are no actual marrow spaces in this region.

The bone which constitutes this sclerotic area consists of two parts, of which one was formed during an earlier period of the disease. This is stained bluish in the specimen stained with hematoxylin and eosin. The other part of the bone, which is pale pink, must have been formed shortly before death. The osteocytes of the bluish bone are crowded and irregular in shape and size; in some places, the bone lacunae are empty, indicating that they are necrotic. The bone is of primitive type and has numerous resorptive cementing lines, an indication that the sclerotic area is the result of vigorous formation, resorption and rebuilding. These resorptive cementing lines are very fine and indistinct, indicating that the resorption and repair followed in rapid sequence. Only in a few places, near the porotic bony area in the upper corner of the picture, does one see dark wide cementing lines, showing that some time elapsed between resorption and rebuilding.

During the last period in the formation of this sclerotic area, resorptive channels were formed within the sclerotic bone, which is very characteristic of type 1. These nourishing channels are bordered by normal lamellated bone. This lamellated bone is pale pink and has fewer lacunas. They are grouped irregularly, but all contain nuclei. In many of these haversian systems the activity of the bone formation ceased some time before death, and therefore the surface of the bone shows a wider and darker resting line. Special attention should be called to the fact that many of these haversian systems are complete all around, which is not in accord with bone alteration characteristic of Paget's disease. Here and there, single osteoclasts and some osteoblasts are found in the haversian channels, but their number is limited when compared with the number of these cells in the zones (*c* and *d* in fig. 2 *A*) showing active Paget's disease where hundreds are seen in a single microscopic field.

The cementing lines being indistinct, the sclerotic bone scarcely shows the characteristic structure of bone involved in Paget's disease. The structure becomes more definite where the staining of the cementing lines is deep blue, as shown near the upper corner of the area.

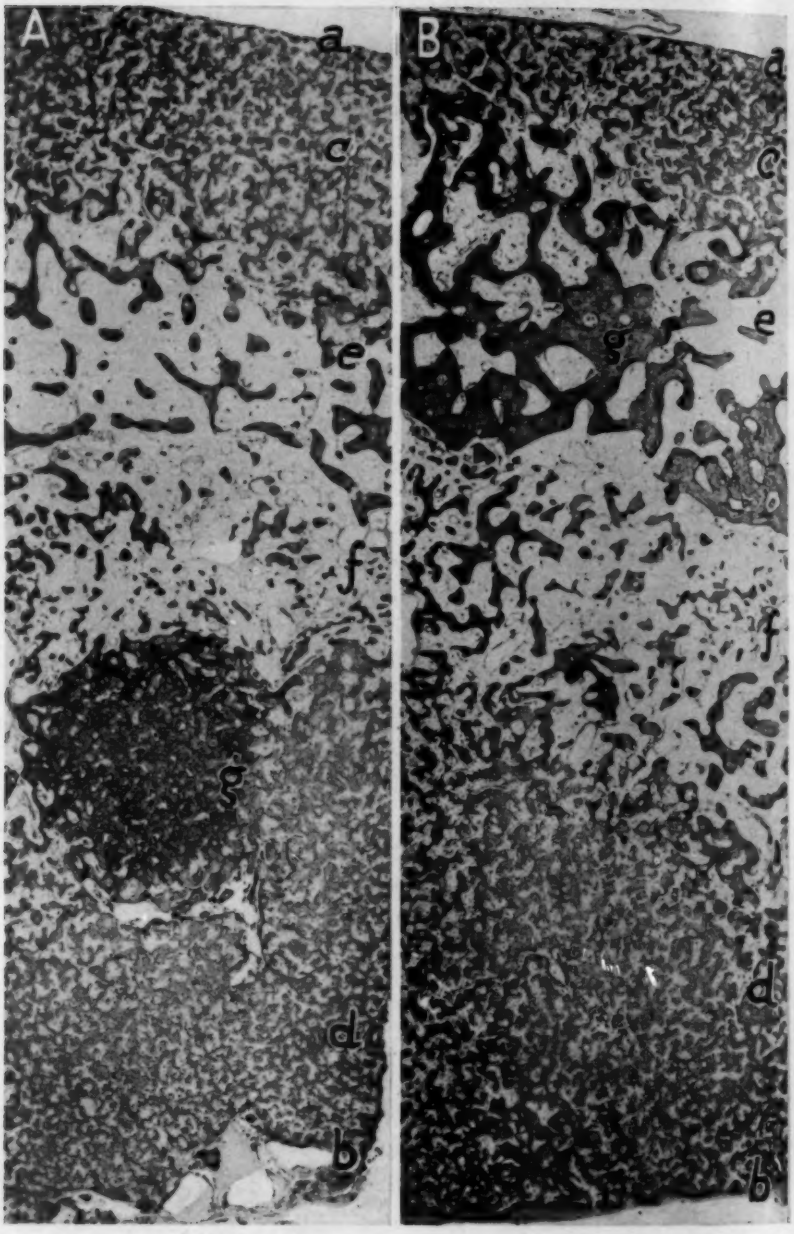

Fig. 2.—*A*, general view of a disk such as that shown in *B* of figure 1: *a*, pericranium; *b*, dura mater; *c*, outer active layer; *d*, inner active layer; *e*, fatty marrow zone; *f*, fibrous marrow zone; *g*, sclerotic body of type 1. *B*, general view of a disk of the cranium involved in Paget's disease, showing a sclerotic area of type 2, *g*, in the fatty marrow zone, *e*. *a*, pericranium; *b*, dura mater; *c*, outer active zone; *d*, inner active zone; *f*, fibrous marrow zone.

As previously mentioned, the section observed in figure 2 *A* shows the characteristic structure of a cranium involved in Paget's disease. Three layers can be grossly distinguished: the external lamina (*c*), the internal lamina (*d*) and the so-called diploe (*e* and *f*). The distinction of external and internal lamina and diploe in the involved cranium is very roughly comparable with that in the normal skull. In this cranium, not a single bone trabecula remains from the preceding normal period. The most active Paget process took place in the outer and inner layers. Here, an unlimited and entirely uncontrolled resorption occurred, and new formation of bone took place simultaneously, so that no haversian systems could develop. Bone was scarcely formed before resorption of some of it set in, and shortly new bone was forming again on the surface of a Howship lacuna.

The diploe is not uniform. A layer of bone can be observed showing fatty marrow (fig. 2 *A*, *e*), with longer and more regular bone trabeculae. Next to this layer fibrous marrow (*f*) can be seen with wide blood vessel spaces and small rounded irregular bone structures. Both these layers in the "diploe" show no resorption and no new bone formation. Neither the bone trabeculae in the fatty marrow layer nor those in the fibrous marrow layer are of normal structure. The bone trabeculae in both layers have a mosaic pattern which indicates the presence formerly of active Paget's disease. In the fibrous marrow the bone trabeculae are surrounded by a dark blue bordering layer, while those in the fatty marrow are bordered at the surface by more regular lamellated layers, which also are in a rest stage. In this zone of fatty marrow the bone trabeculae show signs of active Paget's disease, later normal bone formation and again rest. Occasionally, a recurrence of activity in these rest zones can be found.

The fibrous marrow of the "diploe" frequently shows a scarlike structure, and often there are signs of osteoporosis. The zone of fatty marrow (*e*) is most frequently found between the outer active zone (*c*) and the zone of fibrous marrow (*f*), but sometimes a zone of fatty marrow (*e*) is found between the fibrous zone and the inner active zone (*d*), as shown in figure 6 *B*. In rare cases the zone of fatty marrow (*e*) is found only between the fibrous (*f*) and the inner active zone (*d*), as shown in figure 4 *A*.

The sclerotic area, shown in figures 2 *B* and 3 *B*, is of a structure quite different from that in type 1. This type of sclerosis will be designated as type 2. The sclerotic area is found in the layer of fatty marrow (*e*) and is characterized by a well defined structure within wide bone trabeculae. The bone within the trabeculae is of a primitive type, but at certain places short systems of lamellas are visible. The cementing lines are stained fairly dark blue at some places and very dark blue at

others. The bone corpuscles are not very numerous, and in places they are necrotic. In the center of the bone trabeculae the mosaic bone was formed during an earlier period of the disease and shows the resorption

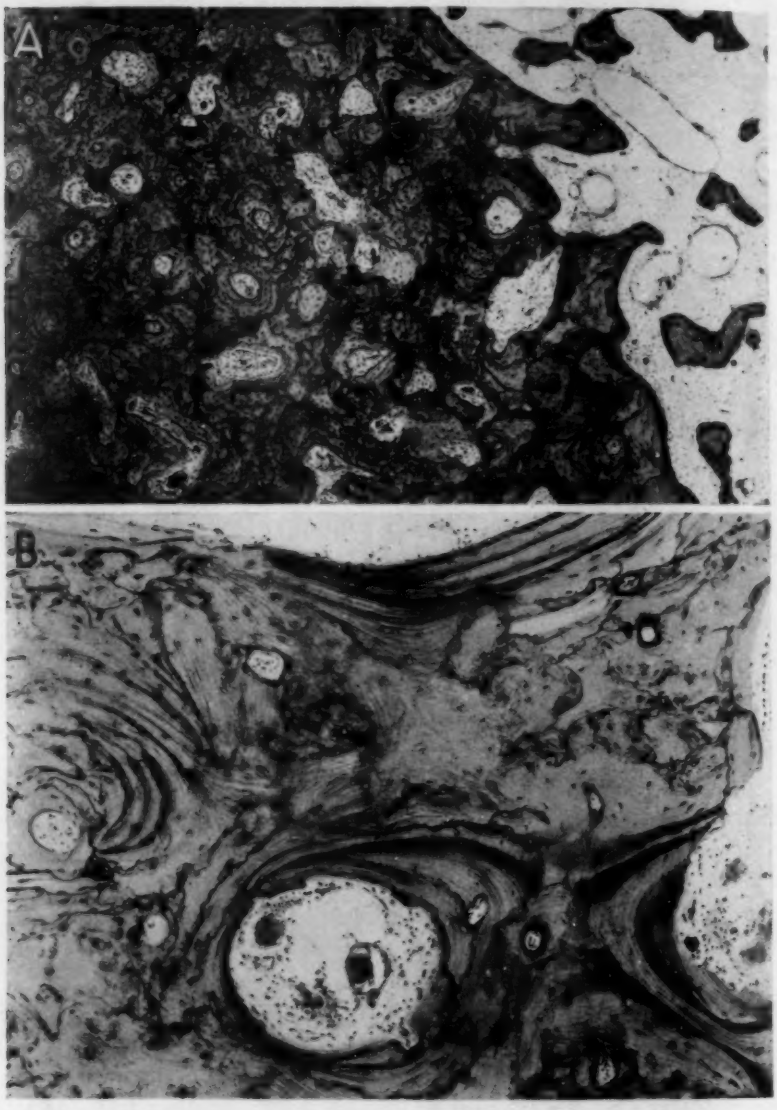

Fig. 3.—*A*, higher magnification of the sclerotic area in *A* of figure 2. Note the compact bone perforated by narrow nutritional channels. *B*, higher magnification of the sclerotic bone in *B* in figure 2. Note the mosaic structure inside the bone trabeculae, the lamellated bone on the surface and the fibrous and the fatty marrow.

and new formation at that time took place in rapid sequence. However, remission occurred, and normal lamellated bone formed on the surface of the mosaic bone. Also complete haversian systems were built. These normal lamellated layers show dark resting lines at some places, indicating slow but regular formation. The lamellated systems were interrupted by resorption at some places but were repaired. Some of these bone lamellas have on their outer surface a fairly wide dark bordering line, which is a sign of a longer remission. Other trabeculae show new osteoblastic bone formation still in progress.

In these sclerotic areas of type 2 the marrow spaces are filled at some places with a loose, soft connective tissue and at other places with a fatty marrow.

The most striking characteristics of the third type of sclerotic area (type 3) are the entire absence of the lamellated haversian systems and the presence of the most primitive bone tissue. Two distinct kinds of bone are found in sclerotic areas of this type; in one the bone corpuscles are very numerous, and in the other they are almost absent. These two kinds of bone can be observed together in figures 4 *A* and 5 *A*. The part which contains very few bone cells has a fibrillated structure and shows only a few, very faint, pale resorptive cementing lines within the bone, but a dark blue finishing line on the surface (*a* in fig. 5 *A*). Most of the bone corpuscles are empty, the bone being necrotic. At some places, a primitive bone formation occurs on the surface of the bone trabecula, where the connective tissue calcifies.

The area of bone containing numerous bone corpuscles shows a large number of very fine, faintly visible cementing lines, most of which are irregular (*b* in fig. 5 *A*). There are no true haversian systems anywhere. The formation of bone takes place largely by calcification of the connective tissue which is in contact with the bone. Cells similar to osteoblasts rarely can be observed bordering the surface of the bone. The bone corpuscles are very irregular in size and arrangement, and most of them are empty. The marrow is a loose connective tissue, not very rich in cells and without special characteristics.

The largest area of sclerotic bone in the skull under discussion may be classified as of this type (type 3). There are sclerotic areas which are difficult to classify as belonging to any one of the specific types. They are mixed types, and their characteristics are less pronounced.

In this cranium the most primitive type of hard tissue formation can be observed in some sclerotic areas. This will be designated as type 4. Figure 4 *B* shows a general view of the structures in question. The sclerotic bone area (*g*) is in a large porotic region within soft connective tissue (*f*). As seen in higher magnification (fig. 5 *B*), the fiber groups

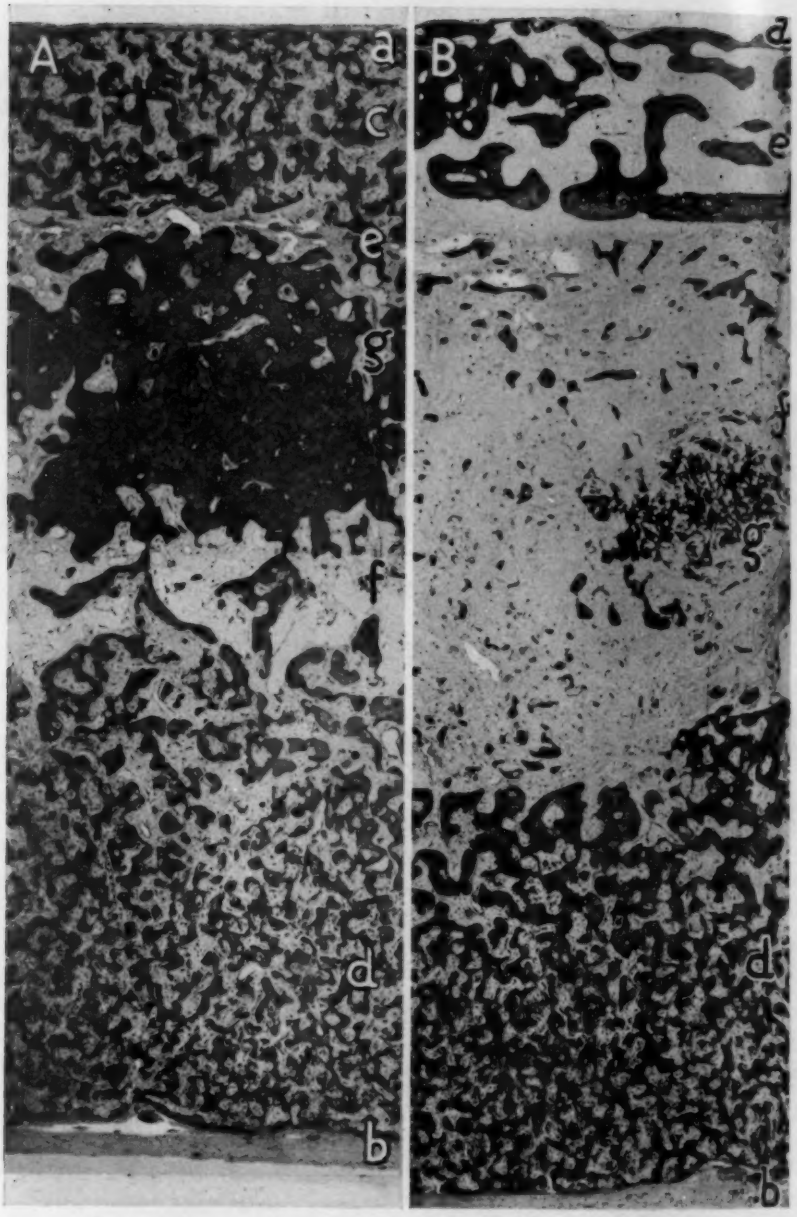

Fig. 4.—*A*, general view of a disk of the skull involved in Paget's disease with a sclerotic body of type 3, *g*, in the fibrous marrow zone, *f*. *a*, pericranium; *b*, dura mater; *c*, outer active zone; *d*, inner active zone; *e*, fatty marrow zone in this section between the fibrous marrow zone and the inner active zone. *B*, general view of a disk of the skull involved in Paget's disease, showing a sclerotic area of type 4, *g*, in the fibrous marrow zone, *f*. *a*, pericranium; *b*, dura mater; the outer active zone is missing; the fatty marrow zone, *e*, is bordering on the pericranium; *d*, inner active zone. The sclerotic area consists of very primitive bone, almost entirely calcified connective tissue.

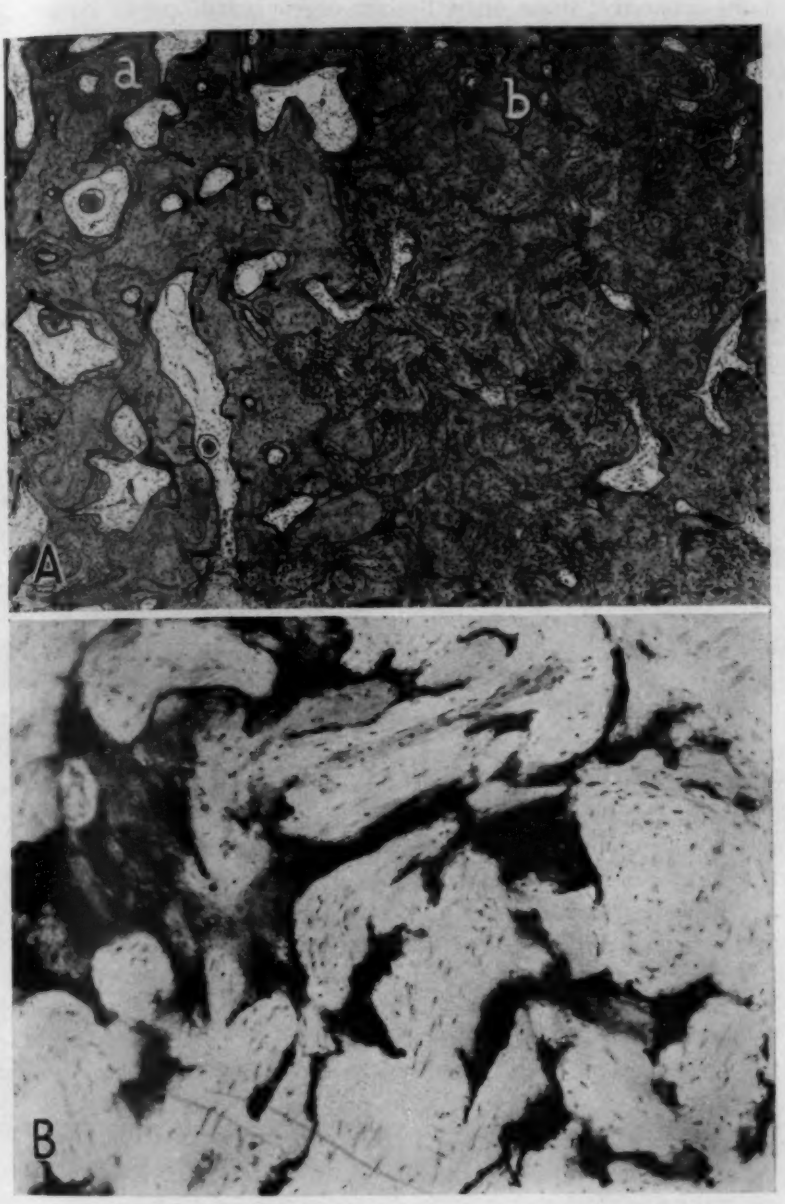

Fig. 5.—*A*, higher magnification of the sclerotic area in *A* of figure 4: At *a*, note the bone with few bone cells, which are necrotic, and the heavy finishing line on the surface of the trabecula; at *b*, bone with very numerous bone cells and a large number of fine cementing lines. *B*, higher magnification of the sclerotic area of *B* in figure 4. Note the calcification of connective tissue forming a sclerotic area.

in the connective tissue show hyaline degeneration, and in these areas of hyaline degeneration calcification takes place. The most primitive bone formation is more aptly called calcification than bone formation. However, in the somewhat wider trabeculae, cell enclosures can be seen with nuclei similar to bone cells. The trabeculae widen and elongate, and the connective tissue surrounding them becomes more and more hyalinized and calcified.

This type of sclerotic bone is seldom found in the pure form but is frequently found together with other sclerotic types, especially types 2 and 3.

Type 5 (fig. 6*A*) is a sclerotic bone area consisting almost entirely of a fine fibrillar matrix, without a definite structure, the cementing lines being entirely absent. The bone lacunas are empty throughout. They are very numerous on the surface, but in some areas within the bone they are scarce. A few narrow nutritional channels penetrate the hard tissue and are bordered by dark limiting lines. Some have been closed by calcification of the connective tissue. Resorption is seen only at one spot on the surface. In some places, progressing calcification of the connective tissue can be observed on the surface. This increases the size of the sclerotic area. The connective tissue surrounding this hard bone island is rich in cells and fibers. Both small and large vessels are present.

The sclerotic area (type 6) shown in figure 6*B* is entirely different in character. This bony area consists of numerous thin, delicate trabeculae, which show a certain grouping. The trabeculae are bordered largely by osteoblasts which seem not to be very active. There are also present resorption lacunas, with and without osteoclasts. The trabeculae show faint cementing lines, giving the appearance of a mosaic structure. It is evident that resorption and bone building periods replaced each other repeatedly. The connective tissue is poor in cellular elements, while fibers are numerous. At some places the connective tissue shows signs of edema. Wandering cells are numerous but show no grouping. The outer and inner layers of the cranial bone (*c* and *d*) show the active stage of Paget's disease. Toward the diploe, which in figure 6*B* is made up entirely of abnormal bone, one finds toward the external and the internal lamina a narrow strip of fatty marrow with wide bone trabeculae.

Concerning the location of the six types of sclerotic bone, the following observations were made: Type 1 was found more frequently at the borderline of the internal lamina and diploe, whereas type 2 was observed more often bordering the external lamina in the fatty marrow zone; occasionally, it filled the entire diploe. Type 3 was located largely in

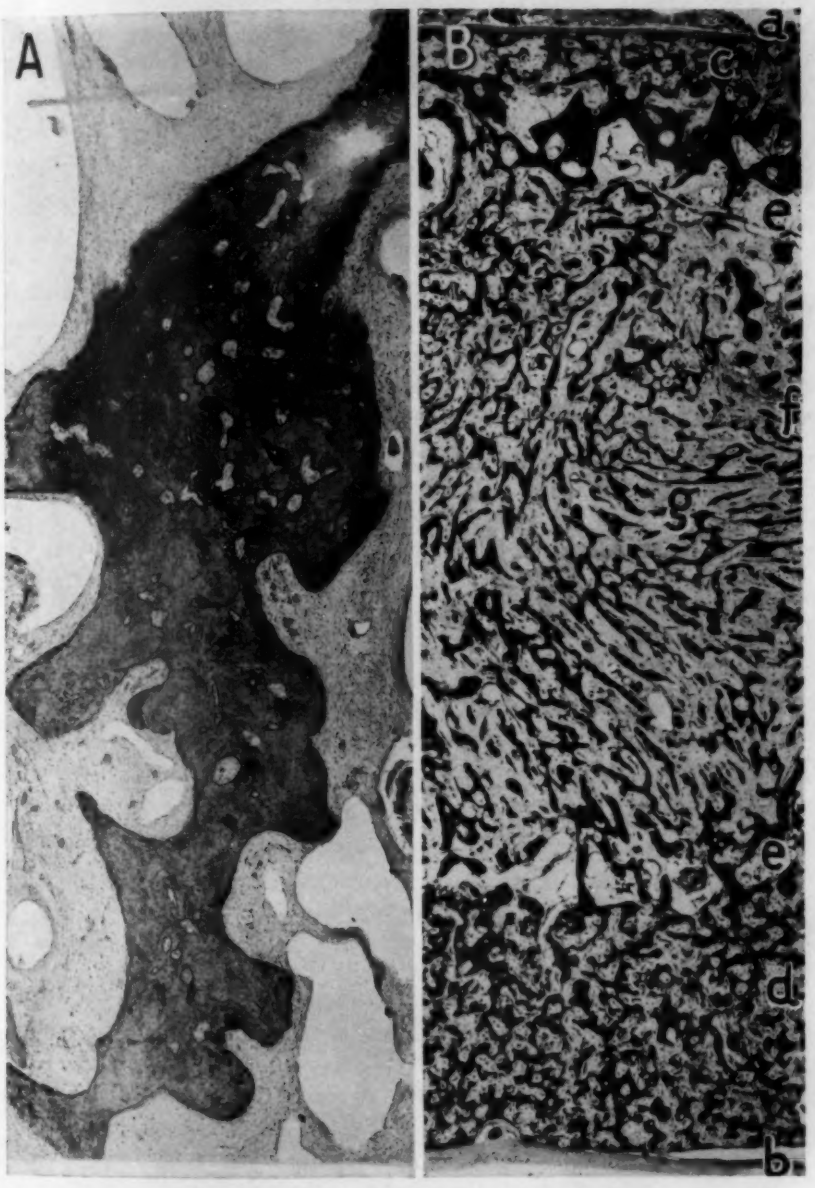

Fig. 6.—*A*, sclerotic body of type 5 in the skull involved in Paget's disease. The body consists of a fine fibrous matrix; cementing lines are missing; the bone lacunae are empty. *B*, sclerotic area of type 6 in the involved skull, showing peculiar fine bone trabeculae, *g*. *a*, pericranium; *b*, dura mater; *c*, outer active zone; *d*, inner active zone; *e*, fatty marrow zone; *f*, fibrous marrow zone.

the fibrous marrow of the diploe. The same applies to type 4, which often accompanied other types. The last two bony structures described were found in the diploe. Two sclerotic areas were found entirely embedded in the internal lamina (*d*). One of them belonged to type 1 and the other to type 2.

In several sclerotic areas, which otherwise showed complete inactivity, recurrence of activity was observed. In these places very active resorption and new formation took place, indicating recurred activity.

SUMMARY

The sclerotic areas which give the roentgenologic picture of the skull involved in Paget's disease its typical appearance are of different structure. Six distinct types are described; the histologic details confirm the characteristic activity in bone involved in Paget's disease, i. e., uncontrolled and repeated alternation of resorption and new formation.

USE OF SERUM ULTRAFILTRATE IN TISSUE CULTURES FOR STUDYING DEPOSITION OF FAT AND FOR PROPAGATION OF VIRUSES

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AND
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The ultrafiltrate from blood serum has been shown [1] to contain a substance (the A factor) which stimulates the growth of dormant adult tissue in vitro and which, furthermore, appears to be essential for the maintenance of living adult cells. This A factor is an acid with a molecule small enough to pass readily through collodion membranes impermeable to proteins. In neutral solution it withstands a temperature of 100 C. but is readily destroyed by heating in dilute acid or alkali.

Serum ultrafiltrate, owing to the presence of this agent, has proved to be a desirable basic medium for tissue cultures. For several years it has been used in the preparation of cultures free from fat granules for the study of fat deposition.[2] Furthermore, it serves as a basic fluid medium in which segments of arteries are incubated for the observation of the fat-depositing action of the B factor [3] and is also used as a standard fluid medium in the study of growth stimulation and inhibition.

More recently serum ultrafiltrate has been adopted as a medium for tissue cultures in which filtrable viruses have been cultivated,[4] with the result that some of these viruses have been obtained in high titers in

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These investigations have been aided by grants from William R. Warner & Company and from the John and Mary R. Markle Foundation.

1. (*a*) Simms, H. S.: Science **83**:418, 1936. (*b*) Simms, H. S., and Stillman, N. P.: J. Gen. Physiol. **20**:649, 1937.

2. Simms, H. S., and Stillman, N. P.: Arch. Path. **23**:316, 1937.

3. Simms, H. S., and Stillman, N. P.: Arch. Path. **23**:332, 1937.

4. (*a*) Sanders, M.: Arch. Path. **28**:541, 1939; (*b*) J. Exper. Med. **71**:113, 1940. (*c*) Sanders, M., and Molloy, E.: Proc. Soc. Exper. Biol. & Med. **45**:327, 1940. (*d*) Molloy, E.: ibid. **44**:563, 1940. (*e*) Jungblut, C. W., and Sanders, M.: J. Exper. Med. **72**:407, 1937.

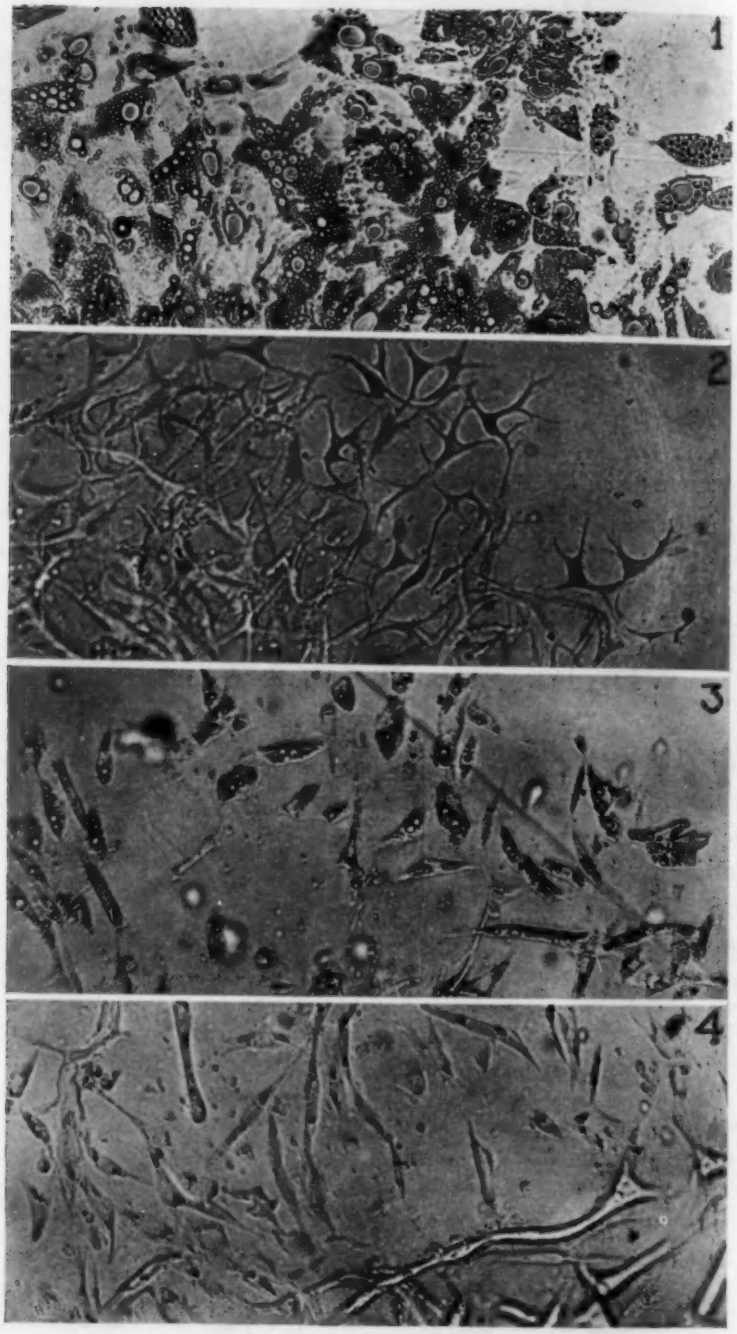

Figure 1

(See legend on opposite page)

a clear fluid free from measurable proteins.[5] The ultrafiltrate is also incorporated in mediums for growing spirochetes [6] and other organisms.

Since various precautions are essential for obtaining satisfactory results, this paper is written to describe the methods which have been developed.

REVIEW OF RESULTS OBTAINED BY THE USE OF SERUM ULTRAFILTRATE

Use in Preparation of Cultures Free from Fat Granules.—Serum ultrafiltrate contains the A factor needed for the maintenance of living cells [7] but does not contain the B factor which causes the formation of fat granules. Hence, fresh cultures of adult chicken fibroblasts can be obtained free from fat granules if they are thoroughly washed with serum ultrafiltrate. These cultures are useful in studying the B factor, which is present in serum and in tissues,[2] and its opposing anti-B factor,[8] which is present in blood serum. The importance of these studies lies in the fact that the B factor has been shown to cause deposition of fat in arteries in vitro and may be involved in the formation of atherosclerotic plaques.

Use of Ultrafiltrate-Washed Cultures for B Factor Testing.—In *1* in figure 1 an unwashed culture is seen to be granular, while the culture shown in *2*, which was prepared as described in the foregoing paragraph, is entirely free from fat granules. When such a culture is incubated with a solution containing the B factor (and ultrafiltrate) it becomes granular [2] as illustrated in *3*. However, if some anti-B factor is present, in addition to the B factor, much less fat is deposited. This is shown in *4*, which may be contrasted with *3*, where the anti-B factor was absent. This method gives a rapid test for these agents which appear to be

5. When made with satisfactory membranes, serum ultrafiltrate contains no protein detectable with trichloroacetic acid. The nonprotein nitrogen was found by Dr. A. A. Albanese (formerly in the department of pathology) to be 15 millimolar. Phosphotungstic acid precipitated 2.6 mM. of nitrogen, of which 1.4 mM. (0.7 m.Eq.) represented basic amino acids while 1.2 mM. was ammonia nitrogen; 12 mM. of nitrogen was not precipitated with phosphotungstic acid.

6. Rosebury, T., and Foley, G.: Proc. Soc. Exper. Biol. & Med. **47**:368, 1941.

7. Work is in progress on a serum globulin fraction which aids in maintaining cultures with little production of fat granules but which is not as indispensable as the A factor.

8. Simms, H. S., and Stillman, N. P.: Unpublished data.

EXPLANATION OF FIGURE 1

Adult chicken aorta tissue cultures (× 175): *1*, culture which was not washed with ultrafiltrate but was allowed to become granular; *2*, culture washed with serum ultrafiltrate; *3*, culture first washed with ultrafiltrate (as in *2*), then treated with B factor; *4*, culture first washed with ultrafiltrate, then treated with B factor plus anti-B factor.

involved in the production of atherosclerotic plaques. The photographs were taken two days after the agents had been added to the cultures. The results are discussed in more detail elsewhere.[9]

Use of Serum Ultrafiltrate as a Fluid Medium.—For a number of years serum ultrafiltrate has been used as a fluid medium for bathing small pieces of tissue in vitro.[10] No plasma clot is present. The tissue merely rests on the bottom of the test tube or other container and is covered with about 100 volumes of dilute serum ultrafiltrate. The ultrafiltrate serves as a basic medium and as a control in testing the stimulating or inhibitory actions of numerous substances. The tissue is afterward planted in plasma medium and its viability determined.

Likewise, in observing the action of the B factor on adult arteries in vitro, segments of these arteries in test tubes are bathed with fluid at 37 C. for seven to ten days. Ultrafiltrate serves as a basic medium and as a control. The tissues when sectioned and stained are found to be in good condition, as was illustrated in a previous paper.[3]

Use of Serum Ultrafiltrate in Cultures for the Propagation of Filtrable Viruses.—Serum ultrafiltrate (diluted with 2 parts of a physiologic solution described in a later section) has been used successfully for the propagation of the viruses enumerated in table 1, in addition to PR8 influenza.[11] The potencies indicated in table 1 were determined by injecting serial dilutions of clear culture fluid into mice, except in the case of lymphogranuloma venereum, in which emulsified whole culture was injected.

The advantages of the use of serum ultrafiltrate for virus propagation are summarized in table 2. The lymphogranuloma venereum preparation is nearly protein-free, causing little or no skin reaction on normal subjects and a definite reaction on those who have had or have the disease. It has considerably less protein than preparations from mouse brain, but the recent antigen of Rake, McKee and Shaffer,[12] prepared from the yolk sacs of fertilized eggs, is also low in protein. Both eastern and western strains of equine encephalomyelitis have been grown by others in physiologic solutions that gave potencies approximately equal to the ultrafiltrate cultures. The advantage of ultrafiltrate cultures lies in their stability as well as in the long survival of the virus. Protein-free fluid from these cultures has proved to be an effective vaccine when tested on guinea pigs. The ultrafiltrate cultures of SK murine poliomyelitis represent a successful cultivation of a neurotropic virus.

9. Simms and Stillman (footnotes 2 and 8).

10. Simms, H. S., and Stillman, N. P.: J. Gen. Physiol. **20**:621, 1937; footnotes 1 *b* and 8.

11. Dochez, A. R., and Mills, K.: Unpublished data.

12. Rake, G.; McKee, C. M., and Shaffer, M. F.: Proc. Soc. Exper. Biol. & Med. **43**:332, 1940.

TABLE 1.—*Viruses Propagated in Serum Ultrafiltrate Tissue Cultures*

Virus	Tissue Substrate	Temperature	Ultrafiltrate Cultures: Activity After Extended Maintenance * 5 Days	10 Days	15 Days	30 Days	Ultrafiltrate Cultures: Maximum Activity
Lymphogranuloma venereum [4b]	Embryo guinea pig brain	Room (21 C.)	10^3	Active	Active	Active	10^3
St. Louis encephalitis [4d]	Embryo mouse brain, minced chick or chorioallantoic membrane †	Room	10^{5+}	10^{5+}	10^{5+}	(10^5)§	10^{5+}
SK murine poliomyelitis [4c]	Embryo mouse brain	Room 37 C.	10^6 10^3	10^6 0	10^5 0	10^4 0	10^6 10^6 1 to 3 days
Equine encephalomyelitis							
(a) Western strain [4c]	Minced chick or chorioallantoic membrane †	Room	10^6	10^5	10^4	...	10^6
(b) Eastern strain [4c]	.....	Room	10^7	...	...	...	10^7

* During the period of extended maintenance the cultures were not transferred or washed.

† Chorioallantoic membrane removed from the egg and placed in serum ultrafiltrate.

§ This value was less accurate than the other values.

TABLE 2.—*Comparison of the Ultrafiltrate Culture Method with Other Methods for Virus Propagation*

Virus	Ultrafiltrate Cultures of Virus: Maximum Activity (Mouse Test)	Protein	Other Preparations of Virus: Claimed Activity (Mouse Test)	Protein	References	Advantages of Ultrafiltrate Cultures
Lymphogranuloma venereum	10^3	Not over 0.2%	10^{3+} *	Low	Rake and others [12]	Ease of preparation
St. Louis encephalitis	10^5	No protein	10^3	Low	Syverton and Berry † Harrison and Moore † Shultz and Williams † Smith, M. G.†	High activity long survival
SK murine poliomyelitis	10^6	No protein	Not grown	..	.....	Not previously grown
Equine encephalomyelitis						
(a) Western strain	10^6	No protein	10^5 Fertile egg preparation	40% protein	Higbie and Howitt † Beard and others †	Low protein and long survival
			10^5 Tyrode culture	Low protein	Cox and others † Olitsky and others †	Long survival
(b) Eastern strain	10^7	No protein	10^9 Fertile egg preparation	High protein	Beard and others †	Low protein and long survival

* The yolk sac titration was 10^8

† Syverton, J. T., and Berry, G. P.: Science **82**: 506, 1935.
Harrison, R. W., and Moore, E.: Proc. Soc. Exper. Biol. & Med. **35**: 359, 1936; Am. J. Path. **13**: 361, 1937.
Shultz, E. W.; Williams, G. F., and Hetherington, A.: Proc. Soc. Exper. Biol. & Med. **38**: 799, 1938.
Smith, M. G.: Proc. Soc. Exper. Biol. & Med. **40**: 191, 1939.
Higbie, E., and Howitt, B.: J. Bact. **29**: 399, 1935.
Beard, J. W.; Beard, D., and Finkelstein, H.: J. Immunol. **38**: 117, 1940
Cox, H. R.; Syverton, J. T., and Olitsky, P. K.: Proc. Soc. Exper. Biol. & Med. **30**: 806, 1933.
Olitsky, P. K.; Cox, H. R., and Syverton, J. T.: J. Exper. Med. **59**: 159, 1934.

DISCUSSION OF TECHNICS OF VIRUS CULTIVATION

As far as we know from our present experience, the most favorable conditions for the propagation of virus parallel the optimum conditions for the maintenance of the tissue in a healthy viable condition.

In the preparation and maintenance of ultrafiltrate cultures for virus propagation there are four points which need particular emphasis: First, the volume of tissue should not exceed 1 part in 100 parts of medium. Second, the p_H should be maintained between 7.2 and 7.6 (preferably at 7.4 or 7.5 as shown by the phenol red indicator). Although cultures can survive at p_H 7.8, their virus potency has been found to be less than at 7.4. This is close to the borderline at which the A factor in ultrafiltrate becomes inactivated, and at which the cultures fail to survive. Third, the p_H should be adjusted with carbon dioxide rather than with hydrochloric acid or with buffer, since the tissues need bicarbonate. This is brought out in table 3. Fourth, to prevent escape of carbon

TABLE 3.—*Growth Stimulation of Serum Adjusted to the Same p_H with Carbon Dioxide as Compared with Hydrochloric Acid* *

Medium Before Planting	Days After Planting		
	2	3	4
Serum + carbon dioxide	1.6	8.7	17.0
Serum + hydrochloric acid	0.1	1.4	3.7

* The values represent the extent of growth on an arbitrary scale.[20]

dioxide, the flasks should at all times be closed with rubber stoppers and not plugged with cotton.

Some investigators have used cotton plugs but have maintained the desired p_H by the presence of excessive amounts of tissue. Such cultures not only lack bicarbonate but also accumulate toxic metabolic products and furthermore have inadequate nutrition, with the result that the yield of virus and the survival of the cultures are diminished.

Although viruses can be propagated in cultures with a medium of X7 physiologic solution, the potencies are lower and the survival is shorter than when ultrafiltrate is present. This is to be expected since the ultrafiltrate provides the A factor which is needed to maintain the tissue cells. On the other hand, dilute serum, although containing the needed A factor, proved to be unsatisfactory since the rapid metabolism of tissue in this medium caused the cultures to become acid and shortened their survival. Heaton [13] observed that embryo tissue under 11 days grows in a saline medium whereas older tissue has other requirements. Serum ultrafiltrate supports embryo as well as adult tissues.

13. Heaton, T. B.: J. Path. & Bact. **29**:293, 1926.

The methods described in this paper relate to the cultivation of viruses in small flasks. We have obtained as good results in the propagation of murine poliomyelitis virus in 500 cc. Erlenmeyer flasks, containing 125 cc. of dilute ultrafiltrate together with a corresponding amount of tissue, as in smaller flasks. The depth of fluid and the volume of air space are not of great importance as long as the p_H is about 7.5 in equilibrium with 5 per cent carbon dioxide, as is shown in table 4.

ULTRAFILTRATE AND SOLUTIONS

The Ultrafilter.—Serum ultrafiltrate is produced[14] with the apparatus shown in figure 2, which is an improvement over the ultrafilter previously described.[1b] Ox serum[15] is used since it is easily obtained and since serum ultrafiltrate contains no species-specific material. Fifteen hundred cubic centimeters of ox serum is placed in each unit of the apparatus and is subjected to a pressure of 600 mm. of mercury by the introduction, from a tank, of nitrogen containing 5 per cent carbon dioxide. The serum is kept in motion along the surface of the collodion membrane[16] by the

TABLE 4.—*Volume of Air* and Depth of Fluid (Dilute Ultrafiltrate) Under Which Adult Chicken Aorta Was Incubated Three Days Before Planting*

Ratio of Air Volume * to Fluid Volume	Depth of Fluid	Ratio of Fluid to Tissue Volume	Relative Growth 4 Days After Planting
1/7	90 mm.	500	32
5	18 mm.	100	33
17	7 mm.	100	37

* By "air volume" is meant the volume of the space between the fluid and the stopper occupied by the air-carbon dioxide mixture.

14. Serum ultrafiltrate is now being produced for experimental purposes by the Warner Institute for Therapeutic Research, 113 West Eighteenth St., New York.

15. Blood is obtained from the slaughter house in sterile cream settling cans. After being kept four or five hours in the ice box, the clot is quartered with a sterile spatula, and a sterile perforated iron weight 3 inches (7.6 cm.) thick is placed on top of the clot. After standing over night, the serum is syphoned off and centrifuged free from red cells. Defibrinated blood may be used, although it is likely to be more hemolyzed. However, hemolysis of ox blood is less objectionable than that of blood from species having a high potassium content in the red cells.

16. The large collodion membranes necessary for this apparatus are made in tubes 380 mm. long with an inside diameter of 38 mm. The open end has a slight bead but no flare. Chemically pure 5 per cent collodion solution (Merck) is poured into each tube up to a 70 cc. mark and allowed to stand a half hour with the top covered, to allow the escape of bubbles. Then a circular piece of paper 82 mm. in diameter, having a 28 mm. hole in the center, is placed over the mouth of each membrane tube, folded down over the sides and held in place with a rubber band.

The tubes are then placed in the membrane machine, where each rests on four no. 3 one hole rubber stoppers, two on each of two ¼ inch (0.64 cm.) steel rods.

(Footnotes continued on next page)

surging action of a rocking trap. Each 1,500 cc. of serum yields 750 cc. (50 per cent) or more of ultrafiltrate within six or seven hours, but twenty hours' filtration is needed to give a 75 per cent yield (1,025 cc.). At the cited pressure the maximum yield [17] is 80 per cent (1,200 cc.). This ultrafiltrate is water clear and should be free from protein. In the event that the membrane is too permeable, a trace of protein and of red color may be present. Such ultrafiltrate is refiltered through another membrane.

Phenol Red.—After the ultrafiltrate has been syphoned from the collecting tube, phenol red indicator is added in suitable concentration. If the ultrafiltrate is to be used in small Carrel flasks or in small test tubes (11 mm. inside diameter), a phenol red concentration of 5 mg. per hundred cubic centimeters is satisfactory. However, for virus work in 50 cc. Erlenmeyer flasks, 1 mg. of phenol red per hundred cubic centimeters gives a more satisfactory color. When larger flasks are used, the suitable concentration of phenol red (in milligrams per hundred cubic centimeters) is approximately equal to 50 divided by the diameter of the flask in millimeters. If merely a thin layer of fluid is used, higher concentrations will be needed.

The phenol [18] red can be made up in a solution containing 500 mg. per hundred cubic centimeters (500 mg. phenol red plus 14.1 cc. of tenth-molar sodium hydroxide plus water to 100 cc.); 10 cc. of this solution in each liter of ultrafiltrate gives a final concentration of 5 mg. of phenol red per hundred cubic

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One of these rods is free to turn while the other rotates at 9.5 revolutions per minute, giving the tube a speed of about 5 revolutions per minute. The surface speed of 65 to 70 cm. per minute is suitable for obtaining good membranes. The rods are carefully adjusted at such an angle that the mouth of the tube is 3 mm. higher than the closed end. There is a 12 inch (30.5 cm.) 40 watt bulb in front of each tube to give heat. One end of the bulb is 15 mm. from the tube near its closed end. The other end of the bulb is 46 mm. from the tube.

After about fifteen minutes' rotation, a hole in the membrane may be seen at the bottom end. In order to close the hole the tube must be removed temporarily and held in a more vertical position (while being constantly rotated at the same speed). The tube is then returned to the machine and left there until a total time of thirty minutes has elapsed (as measured with an interval timer). The tube is then removed and filled with 50 per cent alcohol for a half hour. The alcohol is replaced with distilled water for another quarter hour or longer. The membrane may then be removed from the tube and stored in a large jar of distilled water. Dr. R. C. Horn, of the department of pathology, kindly determined the permeability of these membranes and found them to be about 23 millimicrons. On distention the permeability may drop to 15 millimicrons.

17. At the maximum yield (V_m), the Donnan pressure is equal to the mechanical pressure. As the filtration approaches this point, it becomes progressively slower, following the equation:

$$k\,t = \log \frac{V_m}{V_m - V_t}$$

in which V_t is the yield at time t, and k is a constant depending on the permeability of the membrane. For a good membrane with the maximum permeability which will retain the serum proteins k equals about 0.067 (when t is in hours), so that the initial rate of filtration is 15 per cent an hour.

18. The phenol red was obtained from the National Aniline & Chemical Company, New York.

centimeters. Two cubic centimeters is used to give 1 mg. per hundred cubic centimeters, and so on.

Sterilization of Ultrafiltrate.—After the phenol red has been added, the ultrafiltrate is adjusted to p_H 7.0 to 7.2 with carbon dioxide, sterilized as soon as possible, then stoppered and stored in the ice box. A sterile 5/3 sintered Jena

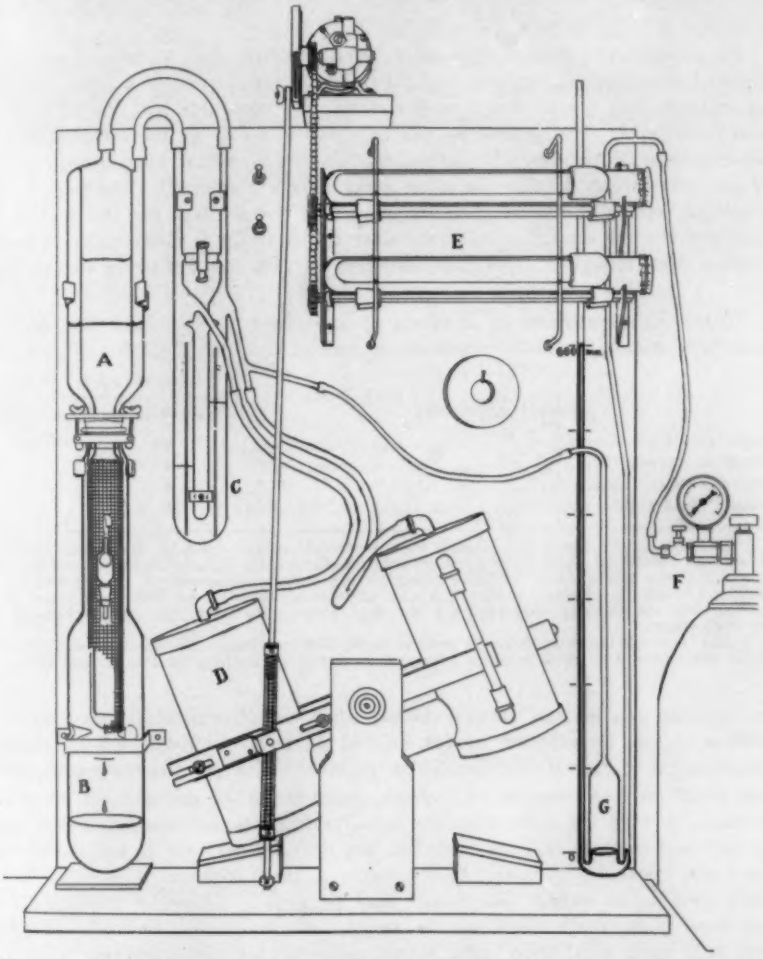

Fig. 2.—Ultrafilter: *A*, ultrafilter "surger tube," having two concentric serum reservoirs; *B*, ultrafilter collecting tube with stainless steel gauze sleeve to protect the collodion membrane; *C*, safety trap with baffle plates, containing sodium bicarbonate buffer with phenol red; *D*, rocking water trap which rocks at 10 cycles per minute; *E*, membrane machine with two membrane tubes; *F*, tank of 95 per cent nitrogen and 5 per cent carbon dioxide; *G*, mercury manometer the scale of which indicates the scale of the drawing.

glass filter is used. Berkefeld and Seitz filters have been found to produce definite toxicity. This is demonstrated with a physiologic solution in table 5. Autoclaving

destroys much of the stimulating activity of the A factor unless the ultrafiltrate is in a sealed container and is cooled rapidly after the sterilization. In the absence of a glass filter, it is possible to sterilize by heating in small containers at 100 C. for ten minutes or at 60 C. for thirty minutes.[19] After such heating, the condensed water at the top of the container should be flamed cautiously to prevent contaminated droplets from running down into the sterile fluid. Larger quantities (500 cc.) can be heated at 80 C. on successive days.

p_H *Adjustment.*—Immediately after being sterilized, the ultrafiltrate should be adjusted, if necessary, to about p_H 7.2 by aseptic introduction of a carbon dioxide-air mixture into the air space in the flask. An apparatus for this purpose has been described.[20] The desired p_H can be obtained either by the introduction of a small amount of 100 per cent carbon dioxide or by several introductions of 7 to 10 per cent carbon dioxide (or other proportion as required). The flask is then stoppered with a rubber stopper and placed in the ice box for storage. It will remain active for a considerable time since the A factor is fairly stable in neutral solution even though it is readily destroyed in acid solution or in slightly alka-

TABLE 5.—*Comparison of Methods of Sterilizing a Physiologic Solution* *

Method of Sterilizing	Growth After 7 Days	
	Experiment 1	Experiment 2
Autoclaved †.....	0 †	0 †
Berkefeld filtration.....	0	0
Seitz filtration.....	0	0.2
Glass (Jena 5/3) filtration.....	15	42

*Portions of the same X6 solution were sterilized by the methods indicated. Pieces of tissue were incubated three days in these solutions and then planted in dilute plasma. The figures indicate the amount of growth seven days later. Analyses of these solutions for sodium, potassium, calcium, magnesium and phosphorus showed no differences which would account for the toxicity displayed by the first three. However, the silicate content was not determined.

† The autoclaving was done in vessels with cotton plugs. Ultrafiltrate autoclaved in sealed containers with rapid cooling appeared to suffer no toxicity or loss of activity.

line solution (p_H 8.0 and above). Later, when the ultrafiltrate is used on tissue cultures, it can be adjusted to any desired p_H by the introduction of whatever concentration of carbon dioxide may be required. Five per cent carbon dioxide is used when p_H 7.4 is desired. The p_H is observed by the color of the phenol red indicator. Should the culture become acid, owing to the metabolism of the tissue, a 2 per cent mixture may be used, but less than 1.5 per cent is inadvisable since the tissue needs bicarbonate. If necessary, a small volume of isotonic fifteen-hundredths molar sodium bicarbonate may be added. Excessive acidity indicates that there is too much tissue for the amount of ultrafiltrate. One part in a hundred is a good proportion since larger quantities of tissue are not adequately nourished.

The reason for using carbon dioxide rather than hydrochloric acid or phosphate buffer is that tissues need some carbon dioxide for adequate growth or maintenance. This was brought out in table 3 where growth was shown to be

19. To facilitate this heating a simple glass reflux bath was designed. This is similar to one made by O. A. Nelson and H. L. Haller (Indust. & Engin. Chem. [Analyt. Ed.] **9**:402, 1937) except that it has a reflux condenser, with down flow cooling, sealed onto the bath. Chloroform is placed in the lower chamber.

20. Simms, H. S., and Stillman, N. P.: J. Gen. Physiol. **20**:603, 1937.

over four times as rapid in the presence of carbon dioxide. It is furthermore essential that ultrafiltrate be kept *stoppered*[21] *at all times* (whether in storage or in use) since the escape of carbon dioxide causes a rise in p_H with resulting inactivation.

Dilution of Ultrafiltrate.—Serum ultrafiltrate is definitely stimulating to adult tissue growth even when diluted to ten times its original volume. However, for good stimulation a dilution to three times its original volume is more satisfactory. This means 1 part of ultrafiltrate plus 2 parts of salt solution (designated UF/3). In this dilution it will maintain living tissue for at least ten days at 37 C. and for longer periods at room temperature. Adult fibroblast cultures have been kept

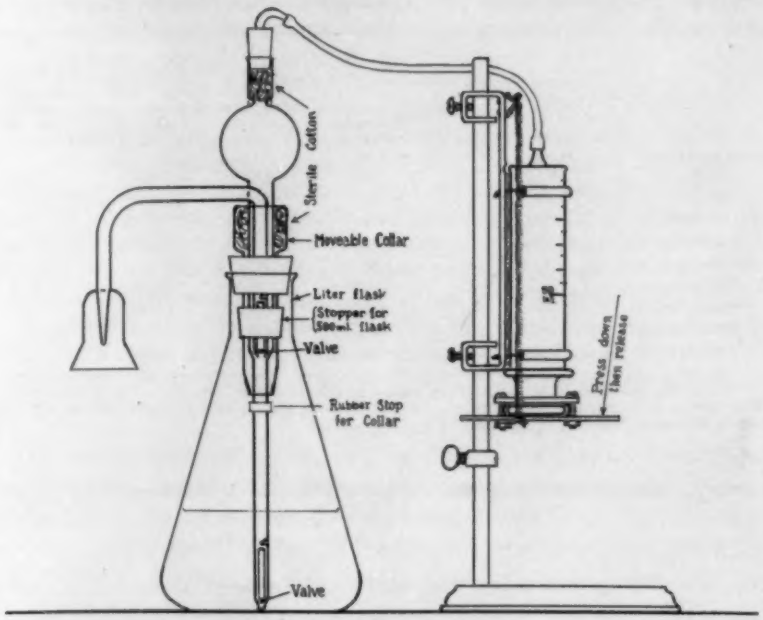

Fig. 3.—Dispensing pump for dispensing measured quantities of sterile solutions from Erlenmeyer flasks.

alive in the same flask without being transferred for one hundred days at 37 C. with UF/3 renewed twice a week.

Dilution is made with the physiologic solution described in the following section. The concentration of phenol red in the physiologic solution is made to correspond with that in the ultrafiltrate to which it is added.

Dispensing the Ultrafiltrate.—The apparatus shown in figure 3 is used to dispense measured volumes of sterile ultrafiltrate or of salt solution. It will be noted that the fluid does not come in contact with rubber. There are no glass joints

21. It is necessary to use rubber stoppers even though rubber is toxic. Fluid which touches a rubber stopper should never be allowed to run down into the flask. A wet stopper should be replaced immediately. These precautions apply to all solutions used in tissue culture.

or stopcocks, and the whole apparatus can be readily sterilized, together with the rubber stopper. When inserted in a 500 or 1,000 cc. flask of fluid, the system is protected from contamination. The connection to the syringe need not be sterile. The syringe is adjusted to deliver any desired volume of fluid up to 50 cc. with an error of less than 0.5 cc.[22] In use the syringe is opened by depressing the handle with the side of the hand. When this is released, a spring closes the syringe and delivers the fluid through the bell.

A similar pump of small size [23] has been used for several years in this laboratory to deliver 0.9 cc. amounts of sterile ultrafiltrate into Carrel flask cultures. The error is less than 0.05 cc.

TABLE 6.—*Composition of the X7 Physiologic Solution Used for Tissue Culture*

	Molecular Weight	20 to 1 Mother Solution, Gm. per Liter	X7 Solution, Final Concentration After Dilution	
			Gm. per Liter	mM. per Liter
Mother solution 1				
$NaCl$	58.5	160.	8.00	137.0
KCl	74.5	4.0	0.20	2.7
$CaCl_2.2H_2O$	147.	2.94	0.147	1.0
$MgCl_2.6H_2O$	203.	4.06	0.203	1.0
Mother solution 2				
$NaHCO_3$	84.	20.2	1.01	12.0
Na_2HPO_4	142.	4.26	0.213	1.5
Dextrose	180.	20.	1.00	5.5
Phenol red *	354.	0.2*	0.01*	0.03*

* When the solution is to be used in small flasks or tubes, the phenol red concentration is five times this amount (designated X6 solution). The suitable concentration for large flasks is discussed under "Ultrafiltrate."

TABLE 7.—*Composition of Mother Solution 3 Which Is Substituted for Mother Solution 2 in Making Z Solution* *

Mother Solution 3	Molecular Weight	20 to 1 Mother Solution, Gm. per Liter	Z Solution Final Concentration After Dilution	
			Gm. per Liter	mM. per Liter
$NaH_2PO_4.H_2O$	138	0.42	0.021	0.15
Na_2HPO_4	142	3.8	0.190	1.35
Dextrose	180	20.0	1.00	5.5

* Z solution contains neither bicarbonate nor phenol red.

Physiologic Solutions.—The solution having the composition given in table 6 is used for diluting serum ultrafiltrate and also for diluting or dis-

22. A dispensing apparatus recently described by N. E. Rigler and G. A. Greathouse (Science **92**:363, 1940) may permit greater accuracy in dispensing large volumes and might be advantageous when precision is required. However, their apparatus involves the sterilization of a glass joint and stopcock, and the fluid comes in contact with a rubber stopper.

23. Both the large and small pumps, as well as the reflux bath,[10] are sold by E. Machlett & Sons, New York.

solving other materials used in tissue culture. This solution (designated by our formula number X7 or X6 according to the amount of indicator used) is a modification of the purgative solution the formula of which was published by Tyrode.[24] It differs from the various Ringer solutions and also from the physiologic solutions of Lewis, Gey and others which have been reviewed by White.[25] The formula of this solution is the result of many tests [8] to determine the optimum concentration of each constituent for growth and maintenance of adult chicken aorta cultures. The low concentration of calcium and the high concentration of phosphate are particularly important. Adult tissue was found to be less sensitive to changes in the concentration of sodium, potassium and chloride. The directions are as follows:

Mother solution 1 is prepared and autoclaved.

Mother solution 2 is made up, acidified with 100 per cent carbon dioxide, then sterilized by filtering through a sintered 5/3 Jena glass filter and kept, stoppered, in the refrigerator. Berkefeld filtration must not be used since it causes toxicity (table 1). Autoclaving is undesirable and should be resorted to only if a glass filter is not available. In the event of autoclaving, the solution must be reacidified with some 100 per cent carbon dioxide under aseptic conditions.[20]

In making up the final solution, 50 cc. of solution 1 is diluted with 900 cc. of freshly redistilled water and autoclaved. When this is cool, 50 cc. of solution 2 is added. The solution is kept in sterile stoppered containers at about p_H 7.4. The p_H may be adjusted by running in enough 5 per cent carbon dioxide to bring the solution to this point.

It is sometimes necessary to expose a physiologic solution to the air. The X solution is not suitable for such purposes since the rapid loss of carbon dioxide causes it to become too alkaline. It is possible to prevent this to some extent by reducing the sodium bicarbonate concentration to half or a third of its normal amount, although this renders the solution less adequate for physiologic purposes.

A solution (designated Z solution) which contains no bicarbonate and hence does not change p_H on exposure to air is made by substituting mother solution 3 (table 7) for solution 2. This is used for temporary bathing or storage of tissues for short periods. It is not adequate for long treatment of tissues, owing to the lack of bicarbonate.

A compromise between the more adequate X solution and the stable Z solution can be obtained by using any desired mixture of mother solutions 2 and 3.

Cleaning of Glassware.—Owing to the fact that 1 part of soap in 1,000,000 will produce fat granules in tissue culture, it is necessary that all glassware which is to be used in the preparation of nongranular cultures should be washed without

24. Tyrode, M. V.: Arch. internat. de pharmacodyn. et de therap. **20**:205, 1910.

25. White, P. R.: Growth **1**:182, 1937.

soap. All our pipets, tubes and small flasks are cleaned by boiling in two-hundredths molar sodium hydroxide followed by two rinsings with distilled water. They are then boiled in hundredth-molar acetic acid and rinsed. Any traces of free acid evaporate on sterilizing. Large flasks and beakers are washed in a dishpan with calgon (but not calgonite, which contains soap), a metaphosphate product, followed by thorough rinsing with tap water, then with distilled water.

For virus cultures these extreme precautions may not be necessary, but are safer. As little soap as possible should be used, and all glassware should be rinsed thoroughly with distilled water.

PREPARATION OF NONGRANULAR CULTURES

Adult chicken aorta is split through the media and the outer half is discarded. The inner half is cut into cubes about 0.6 mm. across, which are planted in Carrel flasks having about 0.05 cc. of fresh dilute chicken plasma (1 part plasma plus 2 parts of X6 physiologic solution) spread over the central portion of the bottom of each flask. The flasks are stoppered and allowed to stand until the plasma is clotted. This part of the technic is the same as previously described[12a] except that only four pieces of tissue are placed in each flask, and no second clot is added. The flasks have a diameter of 30 mm.

We then add to each flask 0.9 cc. of a solution containing 1 part of fresh chicken serum, 2 parts of ultrafiltrate and 5 parts of X6 solution. Each culture is adjusted to p_H 7.4 by the introduction of sterile 5 per cent carbon dioxide in air.[30] The stoppered flasks are placed at 37 C. and are examined with the microscope each successive day.

It will be noted that serum, in a dilution of 1 in 8, is used on new cultures in spite of the fact that serum contains the B factor which causes the formation of fat granules. The serum is added because it contains a protein fraction (associated with the globulins) which is stimulating to growth. As soon as growth is well under way the serum is washed out, as will be described. In order that the concentration of the B factor may be kept at a minimum, the serum should be fresh and should be kept cold until used. A very small amount of spleen extract can be added to clot the fresh plasma in making the serum. Unfortunately, the serum from some chickens is high in B factor, and it is sometimes advisable to use an earlier serum which has been found to be particularly low in B factor.

Washing Cultures with Serum Ultrafiltrate.—After two or three days the cultures begin to put out new cells. When there are 20 to 50 new cells in the clot surrounding at least one piece of tissue in each flask, the cultures are ready for washing. The old fluid is removed from the flask, by suction through a hypodermic needle, and the flask is rinsed with 0.05 cc. of UF/3 (1 part serum ultrafiltrate plus 2 parts X6 solution). This is immediately drawn off and 0.9 cc. of UF/3 is added. A dispensing pump similar to that in figure 3 but of smaller size[23] is used for adding the ultrafiltrate. The p_H is adjusted to about 7.8 by use of a 2 per cent carbon dioxide–air mixture. The stoppered flasks are placed at 37 C. for six hours. A second rinsing and washing are then performed. After another twenty-four hours at 37 C. the new cells may be clear and free from fat granules. If so, they are then ready for use in testing the activity of preparations of B factor or of anti-B factor.

However, if there are still some fat granules a third washing may be needed. Failure to obtain nongranular cultures after three washings may be due to excessively high B factor activity of the plasma or serum used in the fresh culture.

On the other hand, it may be due to the presence of a trace of soap. Soap produces the same effect as B factor and 1 part in 1,000,000 will produce detectable fat granules.

It is necessary to distinguish fat granules (which can be stained [26] with scarlet red) from nonfatty granules or from vacuoles. With a little experience, these can be distinguished without staining. Under low or medium magnification rounded cell debris sometimes gives a culture the appearance of being granular, while high magnification shows that there are no granules in the cell cytoplasm.

It will be noted that the fresh cultures are grown at p_H 7.4 but that during the washing with ultrafiltrate the p_H is raised to about 7.8. This is because the activity of the B factor is less at the higher p_H and the cells at this stage are quite susceptible to the action of the small amount of B factor remaining in the cultures.

Maintenance of Cultures.—We regret that, although we can keep these cultures alive for a considerable time in thick clots, it is difficult to maintain thin clot cultures in sufficiently good condition for testing purposes. As soon as the cultures are ready for testing, the ones which are not used immediately are adjusted to p_H 7.5 (4 per cent carbon dioxide) and placed at room temperature, the ultrafiltrate being renewed twice a week. They may remain in good condition for one week or even two weeks, but degeneration usually sets in before the end of this time. Slightly degenerated cultures sometimes revive on incubation for twenty-four hours. Ice box temperature has not proved advantageous over room temperature. The maintenance of good cultures requires experience.[20]

PROPAGATION OF FILTRABLE VIRUSES IN ULTRAFILTRATE CULTURES

The technic which we have followed in making serum ultrafiltrate cultures has been the same for all viruses except that the source of embryonic cells has varied, depending on the virus under investigation (table 5).

The procedure is as follows:

1. The desired embryo organs are removed aseptically and washed twice in 15 cc. of Z solution (preferably containing 1 part in 4 of ultrafiltrate without phenol red). About 0.33 to 1 cc. of tissue is then placed in a Petri dish containing 3 cc. of fresh ultrafiltrate in Z solution and minced with curved scissors (Mayo or strabismus) into pieces about 0.5 to 1 mm. in diameter with no pieces exceeding 2 mm. Larger pieces of tissue inhibit intracellular diffusion of the

26. The tissue cultures are stained for fat without being removed from the flasks. The flasks are first rinsed then allowed to stand with 50 per cent alcohol for ten minutes, followed by 60 or 65 per cent alcohol for five minutes. Longer fixing is required for thick clots. Two or three drops of scarlet red solution (equal volumes of acetone and 70 per cent alcohol, saturated with scarlet red) are dropped slowly on each colony to be observed and are then allowed to drain off as the flask is inverted for observation. This is a quick method but offers a danger of dissolving the more soluble fat granules. Another method is first to fix with alcohol as described, then to fill the flask with 65 per cent alcohol saturated with scarlet red. The flask is allowed to stand over night. This gives lighter staining but does not remove the fat.

mediums and increase the probability of central necrosis with subsequent production of toxic substances deleterious to both cells and virus. The tissue should not be exposed to the air more than necessary. The minced tissue is again washed twice with 15 cc., then suspended in two volumes of ultrafiltrate in Z solution with the Petri dish tilted. The tissue suspension is now ready to be added to the fluid medium.

Two large drops of the suspension from a capillary pipet contain 0.02 cc. of tissue, which when added to 8 cc. of medium gives a ratio of tissue to fluid of 1 to 400. Experience in growing and maintaining cultures has shown that each volume of tissue needs at least 100 volumes of medium for adequate nutrition. This was borne out in virus cultures by the finding that best virus potency, as well as p_H stability, was obtained when the ratio of tissue to fluid did not exceed 1 in 100. This is especially true of the rapidly metabolizing minced chick cells.

2. About fifty sterile 50 cc. Erlenmeyer flasks, having cotton plugs surrounded by gauze, are assembled.

3. Into each flask 8 cc. of dilute serum ultrafiltrate (1 part ultrafiltrate plus 2 parts X7 solution) is delivered from the dispensing pump shown in figure 3. As this is done, each flask is closed with a no. 2 sterile rubber stopper instead of the cotton plug. This is to prevent the p_H of the ultrafiltrate from rising as a result of the escape of carbon dioxide.

4. The flasks are next opened individually while 2 drops (0.06 cc.) of the tissue suspension are added.

5. Before each flask is closed, 5 per cent carbon dioxide (in air) is introduced aseptically by means of the apparatus previously described.[20] Then the stopper is quickly inserted, and the flask is capped with tinfoil or paper. The p_H should be about 7.5; if not that, it should be readjusted by the introduction of a suitable concentration of carbon dioxide.

6. The cultures are incubated at 37 C. for twenty-four hours.

7. The cultures are removed from the incubator to be inoculated with virus. If not inoculated immediately, they are kept at room temperature in the dark until used.

8. The cultures are inoculated with 0.1 cc. of material containing active virus. Before the stopper is replaced, it is well to introduce more 5 per cent carbon dioxide. Whereas this inoculum in the case of SK murine virus normally contains 3,000,000 mouse paralytic doses, it has been found that an inoculum of less than 10 paralytic doses is not only adequate for the propagation of virus but also results in an equally high potency (10^6 dilution activity, approximately equal to 3×10^8 paralytic doses).

9. The cultures are usually left at room temperature in the dark until transferred or tested for virus content, except for poliomyelitis cultures, which are incubated at 37 C. The clear supernatant fluid then contains the virus which has been propagated.

SUMMARY

The present methods of making serum ultrafiltrate which is suitable for tissue culture mediums are described.

An improved physiologic solution is presented.

Serum ultrafiltrate is used for preparing cultures free from fat granules. These cultures make possible the study of fat deposition in

cultures of adult arterial tissue. This deposition is produced by the B factor present in serum and tissues and is prevented by an anti-B factor present in serum.

Serum ultrafiltrate can be used either as a fluid medium or as a fluid for washing cultures having a plasma clot.

Methods are given for the propagation of viruses in fluid ultrafiltrate cultures which are stable for two weeks or longer. The resulting virus preparations are essentially free from protein yet have high potencies.

Several precautions are emphasized, especially (1) the restriction of the amount of tissue to less than 1 per cent of the fluid volume, (2) the desirability of maintaining the p_H at 7.4 or 7.5, (3) the use of carbon dioxide for adjusting the p_H and (4) the stoppering of flasks to retain the carbon dioxide.

EXPERIMENTAL PEPTIC ULCERATIONS BY VASOMOTOR EPISODES (PITRESSIN EPISODES) AND AUTONOMIC DISTURBANCES

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The extensive literature on the genesis of peptic ulcers presents a number of divergent points of view. Experimentally, interference with the factors on which the vitality of the mucosa is normally dependent, i. e., secretion, circulation and innervation, has given rise to erosions and ulcers, and clinically when peptic ulcers develop it is reasonable to suppose that these factors are affected.

From the literature it appears that local circulatory disturbances produced in various ways are an important factor in initiating peptic ulcers. Disturbed innervation, the neurogenic factor, among others, plays a part not only in the initiation of some of the vascular changes but also in the exacerbations and resulting chronicity of the lesions.

Virchow [1] first laid emphasis on the possible relationship of local circulatory disturbances to peptic ulcer. He noted hemorrhagic necrosis of the mucosa preceding ulceration, which he attributed to vascular spasm. The importance of arteriosclerotic changes in the older age groups has been pointed out by a number of writers. Aschoff [2] expressed the belief that local spasm of the arteries of the stomach is an important factor in the development of erosions, particularly along the gastric pathway.

Anatomically it has been pointed out that the areas in which the greatest number of ulcerations are found have a deficient circulation, i. e., the areas immediately proximal and distal to the pylorus.[3] The arteries of the submucosa in the pyloric region of the inferior portion of the lesser curvature are practically terminal vessels and are sparsely distributed, thus giving a limited blood supply to this area.[4] These arteries are subject to powerful and repeated constrictions by the musculature of the stomach.

From the Department of Pathology, Bacteriology and Public Health, University of Illinois College of Medicine.

1. Virchow, R.: Virchows Arch. f. path. Anat. **5**:632, 1853.
2. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, 1924.
3. Einhorn, M.: Surg., Gynec. & Obst. **50**:416, 1930.
4. Reeves, T. B.: Surg., Gynec. & Obst. **30**:374, 1920.

The occasional association of an intracranial tumor or an ulcer has been recorded. Experimentally, intracranial lesions have led to ulcerations of the stomach and duodenum. Stimulation of various centers has also led to ulcerations. Stimulation of the sympathetic and of the vagus nerves, too, has been associated with gastric lesions. The general ideas of Eppinger and Hess [5] on vagotonia were fostered by von Bergmann,[6] who directed attention to the anemic patches and subsequent ulcerations that occur on occlusion of the vessels by intense muscle spasm. In later papers, while disclaiming that hypervagotonicity is the cause of all ulcers, he asserted that such lesions are more common in persons with a neuropathic constitution—the asthenic, slender habitus. Robinson and Brucer,[7] in a statistical study on a large group of patients with ulcers, pointed out that a statistically significant association was found between slender habitus and ulcers.

In this study the influence of vascular spasm and relaxation as evoked by pitressin in the presence of disturbed innervation as compared with intact innervation on the production of peptic ulcers was investigated.

Because of the conflicting reports in the literature on gastric motility, it was decided to study also the influence of pitressin on the stomach and duodenum.

EXPERIMENTAL OBSERVATIONS

For the study of the effect of pitressin on gastric motility, 12 dogs of approximately the same size and weight were chosen. After a period of training they were subjected to repeated fluoroscopic examinations both before and after operation. In 6 dogs the vagus nerves were cut as they emerged through the diaghragm. In the other 6 both the splanchnic and the sympathetic nerve chains were severed through the abdominal approach. The dose of pitressin injected with certain variations was 20 units per 5 kilograms (following the technic of Nedzel [8]). A third group of 6 normal dogs were used as controls.

The reaction to the dose of pitressin just given varied somewhat from time to time in the same animal and from animal to animal.

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5. Eppinger, H., and Hess, L.: Vagotonia, New York, Nervous and Mental Disease Publishing Company, 1915.

6. von Bergmann, G.: München. med. Wchnschr. **60**:169, 1913; Berl. klin. Wchnschr. **50**:2374, 1913; **55**:524, 1918; Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1923, supp. 4, p. 20; Pathologische Physiologie, Berlin, Julius Springer, 1932.

7. Robinson, S. C., and Brucer, M.: Am. J. Digest. Dis. **7**:365, 1940.

8. Nedzel, A. J.: (*a*) Proc. Soc. Exper. Biol. & Med. **34**:150, 1936; (*b*) Arch. Path. **26**:988, 1938.

The initial reaction in all three groups of dogs was dilatation of the stomach with complete cessation of peristaltic activity. This period was shortest for the vagotomized group (average, seven minutes), slightly longer for the normal group (average, eleven minutes) and markedly increased in the sympathectomized group (average, eighteen minutes). In all three groups, following the initial inhibition, a marked increase in the amplitude and the force of peristalsis occurred. It was probably the most severe in the vagotomized dogs, as their stomachs emptied at a slightly faster rate than the stomachs of normal dogs. In the sympathectomized dogs the augmentation was considerably diminished as compared with that in the other two groups.

In order to determine whether the immediate cessation of peristaltic movements following the injection of pitressin might not be due to excitement, injections of equivalent amounts of saline solution were made. These failed to produce observable changes in peristalsis. With the injection of minute doses of pitressin, from 0.01 to 0.15 cc. (0.2 to 3 units), only inhibition of peristalsis occurred, lasting four to seven minutes, without subsequent increase in peristaltic movements.

Following the intravenous injection of pitressin, a marked spasm of the mucosal vessels (blanching) occurred, which was followed by vascular dilatation of a rather marked degree; then in scattered areas superficial erosions and edema appeared. The experimentally induced disturbance in innervation showed no appreciable effect on the reaction, with the exception that in the sympathectomized dogs the gastric reaction was less severe and erosions following the injections were more infrequently seen. The gross and microscopic observations in the normal dogs were similar to those reported by Nedzel [8] and Metz and Lackey.[9]

The results of repeated injections of pitressin were studied on three groups of dogs. The dogs were approximately of the same size and weight. Dodds and co-workers [10] reported ulcerations in rabbits, cats, monkeys, guinea pigs, rats and mice following injections of an acetone-trinitrophenol extract of the posterior lobe. Nedzel [8b] reported ulcerations of the stomach in normal dogs following injections of pitressin, and Berg [11] reported ulcerations in vagotomized dogs.

In the first group (12 dogs) pitressin was injected intravenously twice weekly. Because preliminary experiments indicated that more than a few injections were required to produce more than a transient erosion, multiple injections were given to produce lesions of greater extent and chronicity. The animals received from eight to twenty injections. In 3 of the 12 animals, erosions and ulcerations were

9. Metz, M. H., and Lackey, R. W.: Texas State J. Med. **32**:589, 1937.

10. Dodds, E. C.; Noble, S. L., and Williams, P. C.: Lancet **1**:1099, 1935.

11. Berg, M.: Am. J. Digest. Dis. **7**:78, 1940.

found. They were mostly superficial and were usually in the pyloric or the fundic portion of the stomach (table 1). Microscopically, they were similar to equal-sized ulcers seen in the vagotomized animals and will be described in detail later.

The second group consisted of 26 dogs of approximately the same size and weight. The vagus nerves were cut as they emerged through the diaphragm, and the operative results were checked at autopsy.

Beginning two weeks after operation, 20 animals were given from two to fifty injections at the rate of two a week (see table 2).

These animals were killed in from thirty to one hundred and fifty days after the onset of the injections. In 13 of the 20 animals erosions and ulcers were found.

TABLE 1.—*Normal Dogs Given Injections of Pitressin*

Dog	Injections	Time	Reactions to Injections	Results
1	8	30 days	Moderate	No gross changes
2	8	30 days	Moderate	No gross changes
3	12	40 days	Severe	Two small ulcers
4	12	40 days	Moderate	Four hemorrhagic erosions near lesser curvature and in pylorus
5	12	40 days	Moderate	No gross changes
6	12	45 days	Moderate	No gross changes
7	16	60 days	Moderate	Numerous small hemorrhagic lesions in pyloric region
8	16	60 days	Moderate	No gross changes
9	16	60 days	Moderate	No gross changes
10	18	60 days	Moderate	Five small ulcerations in stomach in pyloric region
11	20	70 days	Moderate	No gross changes
12	20	70 days	Moderate	No gross changes

Eight injections given over a period of a month were found to produce ulcers in 5 of 6 dogs. In 5 of the dogs the ulcers appeared either in the pyloric region or in the lower portion of the fundus.

Four dogs were killed after receiving ten injections over a period of forty days. They had from five to seven ulcers, all located in the pyloric and the isthmic region of the stomach. The ulcers were larger and deeper than in the previous group.

Eight dogs were killed after receiving fourteen to fifty injections of pitressin and from forty to one hundred and fifty days after the onset of the injections. They were in good health and well nourished with the exception of dog 14, which showed a superficial gastric erosion, and dog 19, which showed hemorrhagic duodenitis; no gross changes were seen in the other animals.

The gross appearance of the earlier lesions may be seen in figure 1. *A* is from dog 5, which was killed after thirty-three days. The lesions are concentrated chiefly along the lesser curvature. In *B* the hemorrhagic duodenitis may be seen.

TABLE 2.—*Vagotomized Dogs Given Injections of Pitressin*

Dog	Injections	Time	Reactions to Injections	Results
1	2	8 days	Very severe	Numerous pinhead to pinpoint size hemorrhagic erosions in pyloric portion of stomach extending up to 5 cm. from sphincter
2	8	30 days	Moderate	No gross changes in stomach or duodenum
3	8	33 days	Moderate	Two small ulcers in isthmus area near lesser curvature and five small hemorrhagic erosions
4	8	33 days	Marked	Small ulcer 1 cm. by 0.5 cm. in a hemorrhagic area near lesser curvature 4 cm. from sphincter; four small erosions in fundus of stomach
5	9	33 days	Moderate	Six small hemorrhagic ulcers in stomach fundus
6	10	40 days	Moderate	Five definite ulcers in pyloric region and fundus
7	10	40 days	Severe	Six definite ulcers in pyloric region and fundus
8	15	50 days	Moderate	No gross changes
9	8	30 days	Moderate	Six hemorrhagic erosions in stomach
10	15	78 days	Moderate	No gross changes
11	10	40 days	Moderate	Four ulcers in pyloric region and three small ones in isthmic region
12	14	65 days	Moderate	No gross changes
13	10	35 days	Severe	Numerous pinpoint to pinhead size erosions in pyloric region of stomach and one larger ulcer
14	25	90 days	Moderate	Small superficial erosion 8 cm. from pyloric sphincter
15	25	90 days	Moderate	No gross changes in stomach
16	4	9 days	Severe	Numerous pinpoint to pinhead size hemorrhagic erosions most severe in duodenum and diminishing as it extends down through the jejunum
17	16	48 days	Moderate	No gross changes
18	25	90 days	Severe	Hemorrhagic duodenitis with grayish adherent patches
19	5	16 days	Severe	Severe hemorrhagic duodenitis and jejunitis as described above
20	50	150 days	Moderate	No gross changes

Acute bloody diarrhea developed in 3 of the animals, and all these died. Two presented almost identical pictures. The intestinal canal was filled with blood, and beginning sharply at the upper end of the duodenum there was marked hemorrhagic duodenitis, which extended down with diminishing severity to the ileum. The third dog presented numerous pinpoint to pinhead–sized hemorrhagic erosions in the pylorus. Of these 3 animals, 1 died of acute hemorrhagic gastritis and 2 of

hemorrhagic enteritis with the greatest involvement in the duodenum. In none of the animals not operated on which were given pitressin did this occur.

The microscopic picture varied. In the superficial acute erosion the mucosa showed evidence of acute congestion, with dilated vessels filled with blood. At the base and the sides of the erosion, small hemorrhages were found. The destruction of the tissues extended to the muscularis mucosae. On the other hand, in other erosions the vascular changes were not so marked. The erosions were found in local areas of edema, which extended on each side from two to three times the width of the lesion. Near some of these, small lymphocytic accumulations were found.

Fig. 1.—*A*, superficial hemorrhagic gastric erosions particularly along the lesser curvature in a vagotomized dog (6) following injections of pitressin. *B*, acute duodenitis with superficial hemorrhagic erosions in vagotomized dog (18) following repeated injections of pitressin.

The deeper ulcers extended down through the submucosa into the muscularis. At the base there was an increase in fibrous tissue, with scattered round cells and scattered dilated vessels; at the margins, fibroblastic proliferation and infiltration by plasma cells. Such an ulcer is shown in figure 2 *A* from dog 6. The lesion extends through the submucosa to the superficial layers of the muscularis. A large thrombosed vessel may be seen to the left.

The microscopic changes in the duodenum were all of an acute nature. The microscopic picture of the acute duodenitis (dog 19) is presented in figure 2 *B*. The many markedly dilated capillaries and

the round cell infiltration near the surface are clearly shown. With slight variations these sections typify the microscopic alterations in the animals presenting duodenitis.

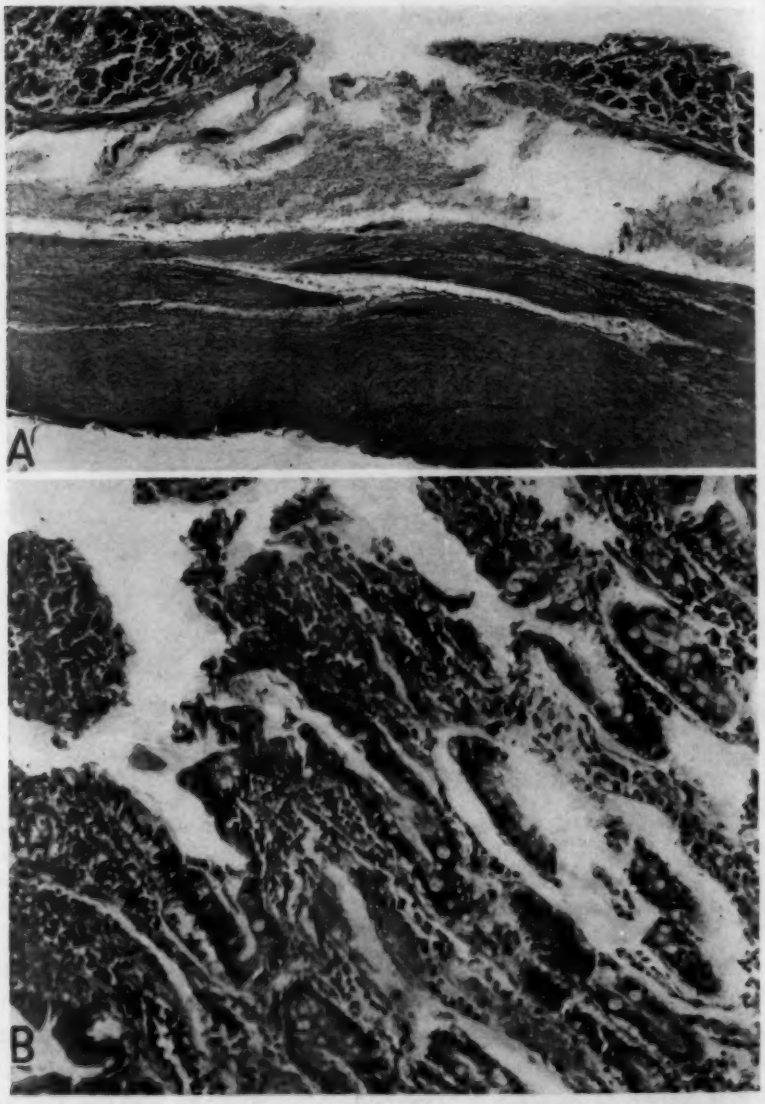

Fig. 2.—*A*, gastric ulcer extending down through the submucosa to the superficial layers of the muscularis, with undermining of the edges by fibroblastic and round cell infiltration. Vagotomized dog 6. × 32. *B*, acute duodenitis showing intense capillary congestion and cellular infiltration. × 110.

In the remaining 6 animals that were vagotomized, but not given pitressin, no evidence of ulceration was found thirty to ninety days

after operation. This confirms the observations of Ivy [12] and others who failed to find evidence of ulceration in vagotomized dogs.

In the third group, consisting of 10 dogs, the splanchnic nerves were sectioned as they came through the diaphragm, many of the fine nerves that enter the adrenals were severed and the sympathetic chain on each side was cut. The operative results were checked at necropsy.

Six dogs were given pitressin in doses as previously described. Three of the dogs were given pitressin twice weekly. Because of the negative results, 2 dogs were given pitressin three times weekly and 1 was given pitressin daily (table 3). In 1 dog a small number of pinpoint hemorrhagic erosions were seen scattered over the first portion of the duodenum. The remaining 4 dogs of this group were used as controls and were not given pitressin. They were killed in from twenty-one to sixty days after operation. In none of this group were lesions demonstrable.

TABLE 3.—*Sympathectomized Dogs Given Injections of Pitressin*

Dog	Injections	Time	Reactions to Injections	Results
1	8	30 days	Moderate	No gross changes
2	12	40 days	Moderate	No gross changes
3	15	45 days	Severe reaction	No gross changes
4	15	45 days	Moderate	No gross changes
5	18	45 days	Severe reaction	A small number of superficial pinpoint hemorrhagic erosions in duodenum
6	10	10 days	Severe reaction	No gross changes

In comparing the normal animals that were given injections in the same manner as the vagotomized and sympathectomized groups it was found that ulcerative lesions were produced in 33 per cent of the first group, compared with 61 per cent of the second group, and with 15 per cent (1 dog) of the third group. The animals not operated on required more injections, and the ulcers were usually not as deep or extensive as in the vagotomized group, although in 1 animal a larger ulcer was seen. In the sympathectomized group, the tendency to ulceration was distinctly diminished, and no gastric lesions were observed.

COMMENT

In the development of the ulcers one notes in both the prepared and the normal groups of animals the initial spasm of the vessels produced by pitressin directly and the spasm produced indirectly as a result of muscular contractions, following which dilatation occurs. In some cases, rupture of a small vessel in the submucosa and superficial erosion occur, or localized areas of edema are found following the ischemia.

12. Ivy, A. C.: Arch. Int. Med. **25**:6, 1920.

These small necrotic areas are digested by the gastric juices, and superficial ulceration may occur. If further spasms do not occur, except as in the particularly reactive animals cited, this lesion heals. With the occurrence of further vascular episodes, the development of ulcers in a certain proportion of the animals follows. The deeper ulcers are similar to those found in man. With the disturbance in innervation from the cutting of the vagus nerves, the ulcers develop more rapidly and more frequently. It appears possible that in the interval between injections the tissues do not recover as rapidly as in the normal dogs, and possibly the spasms are more severe. Aschoff [2] pointed out that ulcers did not heal as rapidly in the vagotomized dogs, and he suggested that the loss of tone of the whole stomach and the decrease in its contractility added considerably to the diminution of the healing tendency. With a disturbance in balance of the vasomotor fibers due to the cutting of the vagus nerves, local spasms [13] leading to impaired vascularity may occur, thus leading to impaired nutrition and diminution of the healing power.

In the sympathectomized dogs the diminished muscular spasm and indirectly the diminished vascular spasm with a resulting lessened tendency to local ischemia and thrombosis may account for the infrequency and the insignificance of the lesions produced.

Long-standing chronic ulcers were not produced. Among the possible factors contributing to this may be mentioned the diminished reaction to pitressin following repeated use, the excellent nutrition of the animals, the gradual resumption of function in the vagotomized animals and what may be cited as the natural tendency for ulcers to heal.

The application of the results of animal experimentation to human pathology is to be ventured only with considerable caution. However, the results may serve to focus attention on certain facts which might otherwise be overlooked or disregarded.

The importance of vascular spasm, not only as produced directly but as caused indirectly, the result of increased muscular constriction, is emphasized by the animal experiments. With disturbed innervation, it is augmented or diminished. Virchow,[1] first, then Aschoff [2] and Smithies [14] (to name but a few of the authors) emphasized the importance of local spasm leading to local anoxia of tissue, predisposing to ulceration and digestion by the pepsin-hydrochloric acid content of the stomach.

It is well recognized that persons subject to ulcers are usually of the asthenic (slender) habitus with labile nervous and vascular systems (Robinson and Brucer [7]). The importance of the vasomotor alterations associated with emotional and anxiety states in aggravating the symptoms of ulcer has been repeatedly emphasized.

13. Dalla Vedova, R.: Policlin. (supp.) **6**:1153, 1900.
14. Smithies, F.: Am. J. Digest. Dis. **2**:437, 1935.

The disease is often seasonal, and Petersen and Milliken [15] have shown that exacerbations are associated with certain meteorologic alterations (polar and tropical fronts).

In the older patients the problem of ulcer is complicated by arteriosclerotic changes and irregular scarring, the result of repeated inflammatory reactions as a consequence of repeated exacerbations.

While emphasis has been placed on the neurovascular mechanism of peptic ulcer, the importance of the other factors mentioned must not be forgotten.

SUMMARY

A single injection of pitressin produces vascular contraction and relaxation with scattered superficial erosions or localized areas of edema. These occur with about the same extent and frequency in normal and in vagotomized dogs. In sympathectomized animals, the reaction is diminished, and erosions or localized areas of edema are infrequent.

Injections of pitressin produce, first, inhibition of gastric and duodenal peristalsis. This period of inhibition is shortest in vagotomized dogs and longest in sympathectonized dogs, normal dogs occupying an intermediate position. This is followed by severe gastric peristalsis in the normal and the vagotomized dogs. In the normal dogs occasional reverse peristalsis is seen, while in dogs with vagus nerves sectioned it has not been observed. In sympathectomized animals the augmentation in peristaltic activities which follows the period of inhibition is distinctly less severe than in normal and in vagotomized dogs. Minute doses of pitressin produce only inhibition in the three groups of animals.

With frequent injections of pitressin, lesions of the stomach are obtained in normal and in vagotomized animals. However, ulcerations are obtained in a greater percentage of the vagotomized group, the lesions are more extensive, and acute hemorrhagic duodenitis is also found. In sympathectomized animals gastric lesions are not obtained. In 1 animal a few superficial erosions were seen in the duodenum.

Microscopically, some of the ulcers observed extended down to the muscularis and resembled those seen in man.

In the groups of animals that were vagotomized and sympathectomized but were not given pitressin, no ulcers were found.

The role of vascular alterations (functional and anatomic), particularly in a subject with a constitutional habitus characterized by increased irritability and vasomotor instability, in the genesis of peptic ulcer is emphasized.

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15. Petersen, W. F., and Milliken, M. E.: The Patient and the Weather, Ann. Arbor, Mich., Edwards Brothers, 1935, vol. 2; 1936, vol. 1, pt. 2; 1938, vol. 4, pt. 3.

FATTY CHANGES IN THE LIVER FROM DIFFERENT CAUSES

COMPARATIVE STUDIES OF THE LIPID PARTITION

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The lipids (neutral fats, cholesterol and phospholipids) are as integral constituents of the cell as the proteins and the carbohydrates. The extent to which these fatty substances occur in a normal cell is subject to a variety of influences and may vary over an appreciable range. The individual lipids may vary in proportion one to another according to the constitution of the individual organism, the state of nutrition and the kind of food intake. Although marked increases of protein or of carbohydrate in the liver do not produce any macroscopic alteration in the appearance of this organ, an accumulation of fat is readily recognized by its yellowish color.

Investigations, therefore, on the abnormal fat content of organs began as early as histologic technic would permit. These studies were, however, necessarily limited to staining procedures. The question with which early investigators concerned themselves was: Did the fat in a fatty organ merely displace the otherwise normally composed cells, or was the fat produced in the cell itself as a result of some intracellular disturbance which produced a change in the proportion of the cell constituents? The former condition was called fatty infiltration to distinguish it from the latter, which was variously designated as fatty degeneration, fatty metamorphosis and "fat phanerosis" (Virchow). Aschoff [1] introduced the expression "fat transformation." He wished by this designation to

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Most of the material used in these studies was supplied by Dr. Sidney Farber, of the department of Pathology of the Harvard Medical School and the Children's Hospital of Boston. Some of the material was supplied by Dr. Timothy Leary, Suffolk County medical examiner. Mr. Joseph Benotti, chemist at the Boston Dispensary, gave technical advice.

1. Aschoff, L.: Vorträge über die Pathologie, Jena, Gustav Fischer, 1925, p. 49.

imply that other cell constituents, such as proteins and carbohydrates, might be transformed into lipids. That conversion of carbohydrate to fat does occur has been proved in numerous experiments in which overfeeding of geese with carbohydrate resulted in extracellular deposition of fat in the liver. It has not been demonstrated, however, that such transformation can occur intracellularly and result in accumulation within that same cell.

The purpose of this study was to investigate the proportions of the various lipids in both normal and fatty livers. In a number of cases, the serum and the spleen from the same person were examined. It was thought that chemical partition of the lipids in fatty livers might reveal characteristic patterns indicative of the mechanism of their origin, i. e., whether, as mentioned in the foregoing paragraph, they are deposited from the blood, or whether they are produced within the tissue cell itself.

The fatty livers examined were from persons who died of the following diseases, and the results of their analysis will be reported in the order given: (1) infectious and consuming diseases; (2) chronic alcoholism with cirrhosis of the liver; (3) toxemia of pregnancy; (4) celiac disease; (5) glycogen storage disease (von Gierke's disease); (6) idiopathic familial hyperlipemia with hepatosplenomegaly, and (7) essential xanthomatosis,[2] Gaucher's disease, Niemann-Pick disease and Tay-Sachs disease.

METHODS

The analytic results which are presented in this paper were compiled after examination of carefully dried, pulverized tissue. The process of desiccation, which is described in a previous paper,[3] consists essentially of immersion of small pieces of the fresh material in solid carbon dioxide-cooled ether; following this, drying is accomplished in vacuo over phosphorus pentoxide. The use of dehydrated material rather than wet tissue is to be strongly advised for the following reasons: Quantitative transfer of precisely weighed samples is greatly facilitated. Since the extraction of lipids from tisue frequently requires the use of solvents which are immiscible with water, greater ease of extraction is afforded than would be possible with the intact, wet tissue. Perhaps of greatest importance is the fact that comparable results are yielded, since the variable due to the water content of fresh tissue is eliminated.

2. "Primary essential xanthomatosis" designates a systemic disturbance in which foam cells may arise in various organs where reticulum cells and histiocytes are present. In this disorder the serum is not milky. Tendon xanthomatosis and the Schüller-Christian syndrome represent the two extremes of this group. The analyzed liver reported was obtained in a case of generalized essential xanthomatosis. "Secondary xanthomatosis" connotes, in contrast to "essential xanthomatosis," a condition in which the presence of foam cells is the result of a dyscrasia characterized by milky serum (hyperlipemia), in which the fatty material is transported by the blood and retained in the reticulum cells (as in diabetes or chronic pancreatic disease). (Thannhauser, S. J.: Lipidosis: Diseases of the Cellular Lipid Metabolism, New York, Oxford University Press, 1940, pp. 48-155.)

3. Thannhauser, S. J., and Setz, P.: J. Biol. Chem. **116**:533, 1936.

With regard to the methods employed, lecithin, cephalin and sphingomyelin were determined as described by us in an earlier publication [4]; free and total cholesterol were estimated by the method of Schoenheimer and Sperry [5] as modified by us. For the determination of total lipid fatty acids, the method of Stoddard and Drury [6] was adapted to the analysis of tissue. Neutral fat was not estimated as such but was arrived at arithmetically. This was done by subtracting from the total lipid fatty acids that quantity of fatty acids which was contributed by both the total phospholipids and the total cholesterol esters; the remainder when multiplied by the appropriate factor yields a value which represents substantially neutral fat. The factors employed for the calculation of phospholipid and cholesterol ester fatty acid were 0.69 and 0.72, respectively. The value for the fatty acids unaccounted for by the aforenamed lipids is converted to the value for neutral fat on multiplication with the factor 1.04. This may be summarized:

> Gm. per hundred grams total lipid fatty acids minus (Gm. per hundred grams ester cholesterol × 0.72 plus Gm. per hundred grams total phospholipid × 0.69) × 1.04 = Gm. per hundred grams neutral fat

The factors given assume an average fatty acid molecular weight of 277.

It may be mentioned at this time that our values for ester cholesterol represent a greater part of the total cholesterol than that indicated by reports of other investigators.[7] We are at a loss to explain this finding. There is a possibility, however, that this may be related to our use of rapidly dried tissue for analysis. The use of wet material introduces the danger of hydrolysis of the cholesterol esters. Such decomposition would account for the preponderance of free over ester cholesterol as found by these workers.

The analytic findings shown in table 1 reveal that in the type of fatty liver associated with infectious diseases and general cachexia there are but negligible departures from normal in the total, the free and the ester cholesterol and the various phospholipids. The only great increase is found in the total fatty acids and consequently in the neutral fat fraction. It is generally believed that the fatty livers observed in cases of infectious and cachectic diseases represent a type of fatty change which is the result of fatty infiltration without much destruction of liver parenchyma. The only distinctive feature, then, of this type of liver is its high fat content.

In the examination of fatty livers in cases of chronic alcoholism (table 2), the most impressive change was found in the neutral fat fraction, which exhibited enormous increases over normal. In marked

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4. Thannhauser, S. J.; Benotti, J., and Reinstein, H.: J. Biol. Chem. **129**:709, 1939. Thannhauser, S. J.; Benotti, J.; Walcott, A., and Reinstein, H.: ibid. **129**: 717, 1939.

5. Schoenheimer, R., and Sperry, W.: J. Biol. Chem. **106**:745, 1934.

6. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1932, vol. 2, p. 499.

7. (*a*) Ralli, E. P.; Rubin, S. H., and Ringler, S.: J. Clin. Investigation **20**:93, 1941. (*b*) Breusch, F., and Scalabrino, R.: Ztschr. f. d. ges. exper. Med. **94**:569, 1934.

TABLE 1.—*Lipid Partition of Fatty Livers Involved in Infectious Diseases and Conditions of General Cachexia*

	Normal Range, Gm. in 100 Gm.	Subacute Bacterial Endocarditis, Gm. in 100 Gm.	Broncho-pneu-monia, Gm. in 100 Gm.	Fibro-caseous Pulmonary Tuberculosis, Gm. in 100 Gm.	Metastatic Carcinoma, Gm. in 100 Gm.
Total cholesterol	2.1 - 2.6	1.98	2.69	2.33	3.03
Free cholesterol	0.44- 0.55	.....	0.36	0.27	0.79
Ester cholesterol	1.50- 2.15	.....	2.33	2.06	2.24
Total phospholipids	9.0 -11.0	7.69	10.6	11.3	9.55
Sphingomyelin	0.3 - 0.5	0.19	0.27	0.68	0.43
Cephalin	3.0 - 5.5	3.74	.....	3.77	Traces
Lecithin	3.0 - 6.0	4.76	.....	6.85	9.12
Total fatty acids	8.6 -13.0	38.5	15.4	17.3	10.4
Neutral fat	1.4 - 4.0	35.1*	6.7	8.4	2.27

* This value is approximate.

TABLE 2.—*Lipid Partition of Fatty Livers in Cases of Alcoholic Cirrhosis, with Comparative Analyses of the Fatty Constituents of Spleens and Serums in Some of the Cases*

		Value in Cases of Alcoholic Cirrhosis					
	Normal Range, Gm. in 100 Gm.	1, Gm. in 100 Gm.	2, Gm. in 100 Gm.	3, Gm. in 100 Gm.	4, Gm. in 100 Gm.	5, Gm. in 100 Gm.	6, Gm. in 100 Gm.
Liver:							
Total cholesterol	2.1 - 2.6	1.13	1.06	0.88	1.68	1.91	1.80
Free cholesterol	0.44- 0.55	0.37	0.24	0.16	0.13	0.18	0.33
Ester cholesterol	1.50- 2.15	0.76	0.82	0.72	1.55	1.73	1.47
Total phospholipids	9.0 -11.0	6.35	4.92	3.58	3.26	4.02	7.34
Sphingomyelin	0.3 - 0.5	0.07	0.04	0.09	0.05	0.12	0.89
Cephalin	3.0 - 5.5	.....	.....	.....	0.18	1.87	1.94
Lecithin	3.0 - 6.0	.....	.....	.....	3.03	2.03	4.51
Total fatty acids	8.6 -13.0	41.6	42.0	57.5	56.4	54.8	25.1
Neutral fat	1.4 - 4.0	38.2	38.0	56.5	65.5	52.9	19.8
Spleen:							
Total cholesterol	0.6- 2.3	.....	.....	.....	2.95	2.22	1.91
Free cholesterol	0.5- 1.1	.....	.....	.....	0.64	0.70	0.89
Ester cholesterol	0.2- 1.2	.....	.....	.....	2.31	1.52	1.02
Total phospholipids	5.5-11.0	.....	.....	.....	8.10	6.35	4.83
Sphingomyelin	0.7- 1.0	.....	.....	.....	1.03	0.37	1.22
Cephalin	1.6- 4.0	.....	.....	.....	5.57	4.03	2.32
Lecithin	3.1- 4.0	.....	.....	.....	1.50	1.95	1.29
Total fatty acids	7.0- 9.0	.....	.....	.....	4.90	4.02	3.86
Serum:							
Total cholesterol	150-230	.....	.....	533.0	232.0	140.0	174.0
Free cholesterol	35- 60	.....	.....	72.0	68.0	89.4	53.9
Ester cholesterol	120-170	.....	.....	461.0	164.0	50.6	120.1
Total phospholipids	175-275	.....	.....	1,310.0	730.0	246.0	286.0
Sphingomyelin	15- 35	.....	.....	40.0	4.3	7.7	15.4
Cephalin	50-130	.....	.....	65.0	501.0	52.0	.....
Lecithin	50-200	.....	.....	1,205.0	224.7	186.3	.....
Total fatty acids	200-400	.....	.....	1,830.0	.....	368.0	314.0
Neutral fat	0-100	.....	.....	618.0	.....	169.0	31.0

contrast to the neutral fat were cholesterol (free and ester) and total phospholipids, the values for which were low. Such decreases may be the result of "dilution," due to the much greater quantities of neutral fat present, a suggestion offered also by other investigators.[7a] There is, however, no proportional decrease in the individual phospholipids. Lecithin, which normally represents approximately 50 per cent of the total phospholipid fraction of liver, may rise to 90 per cent in extremely fatty livers of this type. Cephalin appears to exhibit one of the most prominent decreases. The abnormal content of lipids in fatty livers in cases of chronic alcoholism is apparently due not only to fatty infiltration but also, to a lesser degree, to a change in the lipid content of the cells of the liver parenchyma.

Lipid analyses were carried out on the spleens and serums in several of these cases. Little of interest was observed as to the spleen, all con-

TABLE 3.—*Lipid Partition of Livers in Cases of Toxemia of Pregnancy*

	Normal Range, Gm. in 100 Gm.	Case 1, Gm. in 100 Gm.	Case 2, Gm. in 100 Gm.
Total cholesterol	2.1 - 2.6	8.00	4.05
Free cholesterol	0.44- 0.55	0.27	1.97
Ester cholesterol	1.50- 2.15	7.73	2.08
Total phospholipids	9.0 -11.0	8.9	10.75
Sphingomyelin	0.3 - 0.5	0.33	.....
Cephalin	3.0 - 5.5	.....	.....
Lecithin	3.0 - 6.0	.....	.....
Total fatty acids	8.6 -13.0	9.84	10.1
Neutral fat	1.4 - 4.0	0.0	1.25

stituents being within reasonably normal limits. Several of the serums, on the other hand, showed marked departures from the normal. The serum in case 3 exhibited hyperlipemia, and not only was neutral fat found to be increased, but cholesterol and lecithin as well. The serums in cases 4, 5 and 6 were normal in physical appearance, and in only a few instances did any of the lipid constituents vary significantly from normal. The serum in case 4 had an unexpectedly high cephalin value (due perhaps to an error of analysis), and the serum in case 5 showed an inverse free cholesterol–ester ratio.

One might expect that, coincident with the deposition of neutral fat in the liver, larger quantities would be encountered in the serum. This was found to be true only in case 3. It may be that fatty infiltration is preceded by transient hyperlipemia and that, although the serum lipids return to normal, the fat already deposited in the liver remains.

In toxemia of pregnancy, the chief increase among the liver lipids is due to cholesterol, this being elevated from two to four times normal. The other lipids are all present in normal quantities (table 3). This change in the composition of the liver lipids is believed to be due not

to extrinsic factors but to degeneration of the liver parenchyma. It is of interest to note that a similar increase of cholesterol is observed in the kidney in cases of so-called fatty degeneration of that organ.

In 2 cases of celiac disease (table 4) the livers, as indicated by gross appearance, were found on analysis to differ greatly in lipid composition.

TABLE 4.—*Lipid Partition of Fatty Livers in Cases of Celiac Disease*

	Normal Range, Gm. in 100 Gm.	Case 1, Gm. in 100 Gm.	Case 2, Gm. in 100 Gm.
Total cholesterol	2.1 - 2.6	2.15	0.14
Free cholesterol	0.44- 0.55	0.42	.....
Ester cholesterol	1.50- 2.15	1.73	.....
Total phospholipids	9.0 -11.0	9.19	2.58
Sphingomyelin	0.3 - 0.5	0.66	0.07
Cephalin	3.0 - 5.5	.....	.....
Lecithin	3.0 - 6.0	.....	.....
Total fatty acids	8.6 -13.0	13.0	51.7
Neutral fat	1.4 - 4.0	5.63	52.0*

* This value is approximate.

TABLE 5.—*Lipid Partition of Fatty Livers in Cases of von Gierckе's Disease, with Comparative Analyses of Serums in Some of the Cases*

	Normal Range, Gm. in 100 Gm.	Case 1, Gm. in 100 Gm.	Case 2, Gm. in 100 Gm.	Case 3, Gm. in 100 Gm.	Case 4, Gm. in 100 Gm.
Liver:					
Total cholesterol	2.1 - 2.6	0.83	0.49	0.23	1.64
Free cholesterol	0.44- 0.55	0.17	0.27	0.18	.....
Ester cholesterol	1.50- 2.15	0.66	0.22	0.05	.....
Total phospholipids	9.0 -11.00	5.42	4.38	2.55	7.85
Sphingomyelin	0.3 - 0.5	0.38	0.38	0.21	Traces
Cephalin	3.0 - 5.5	3.42	.....	0.29	Traces
Lecithin	3.0 - 6.0	1.62	.....	2.05	7.85
Total fatty acids	8.6 -13.0	22.6	34.2	52.4	34.2
Neutral fat	1.4 - 4.0	19.1	32.2	52.6	30.0*
Glycogen	.....	36.6	12.7	27.0	8.5
Serum:					
Total cholesterol	150-230	.....	532.8	412.0	.....
Free cholesterol	35- 60	.....	272.0	240.0	.....
Ester cholesterol	120-170	.....	260.8	172.0	.....
Total phospholipids	175-275	.....	832.5	650.0	.....
Sphingomyelin	15- 35	.....	.....	14.0	.....
Cephalin	50-130	.....	.....	62.0	.....
Lecithin	50-200	.....	.....	573.7	.....
Total fatty acids	200-400	.....	4,915.0	1,820.0	.....
Neutral fat	0-100	.....	4,320.0	1,300.0	.....

* This value is approximate.

In case 1 the lipids were found to be essentially normal, in contrast to those in case 2. The latter exhibited a great increase in the neutral fat fraction, the cholesterol and phospholipids being relatively reduced. The disparity in lipid content of these livers suggests that the underlying pathologic changes might not have been the same in each case. However, histologic examination of the pancreas by Dr. Sidney Farber, of the department of pathology of the Children's Hospital, Boston, revealed

fibrosis in both cases. Since in celiac disease the degree of pancreatic fibrosis may differ, the liver fat may likewise be expected to vary.

In cases of von Gierckе's disease (table 5) chemical examination of the liver revealed that the lipids follow the same general pattern noted in association with chronic alcoholism, general cachexia and celiac disease. In each instance there was observed a proportionate reduction of cholesterol and phospholipid, probably due to the increased bulk of neutral fat. In livers 2 and 3 there was an inverse free cholesterol–ester ratio, a finding which interestingly enough was also observed in the two corresponding serums. As is well known, glycogen was found in considerable quantities.

In contrast to the livers with their low cholesterol and phospholipid values, the serums in these cases were found to contain markedly

TABLE 6.—*Lipid Partition of Serum and Liver in a Case of Idiopathic Hyperlipemia*

	Range for Normal Liver, Gm. in 100 Gm.	Liver in Case of Idiopathic Hyperlipemia, Gm. in 100 Gm.	Range for Normal Serum, Mg. in 100 Gm.	Serum in Case of Idiopathic Hyperlipemia, Mg. in 100 Gm.
Total cholesterol...	2.1 - 2.6	2.52	150-230	379.0
Free cholesterol...	0.44- 0.55	0.73	35- 60	158.5
Ester cholesterol...	1.50- 2.15	1.79	120-170	220.5
Total phospholipids	9.0 -11.0	10.05	175-275	465.0
Sphingomyelin	0.3 - 0.5	1.44	15- 35	12.6
Cephalin	3.0 - 5.5	3.05	50-130	Traces
Lecithin	3.0 - 6.0	5.56	50-200	452.4
Total fatty acids...	8.6 -13.0	9.45	200-400	3,115.0
Neutral fats.....	1.4 - 4.0	1.22	0-100	2,740.0

increased quantities of these lipids. Both serums were milky, serum 2 having a neutral fat content of more than forty times normal.

A comparison of the hepatic and serum lipids in this disease is of great interest. It illustrates that the liver cholesterol and phospholipids are not necessarily influenced by an abundance of these lipids in the serum. This observation suggests that there may exist a regulatory mechanism having influence over the quantity of each lipid to be retained by that organ.[8]

It was previously stated that the retention of neutral fat by the liver may be controlled by some regulatory mechanism.[8] This belief is supported impressively by a comparison of the serum and liver lipids in a case of idiopathic hyperlipemia (table 6). Macroscopically the liver presented a perfectly normal appearance; even histologic examination failed to reveal an increase of neutral fat.[9] These observations were borne out by chemical analysis. As may be seen, the hepatic lipids were

8. Dragstedt, L. A.; Van Prohaska, J., and Harms, H. C.: Am. J. Physiol. **117**:175, 1936.

9. Goodman, M.; Shuman, H., and Goodman, S.: J. Pediat. **16**:596, 1940.

essentially normal. The serum, on the other hand, presented a striking aberration from the normal. Intensely milky, it contained approximately thirty times the normal quantity of neutral fat. The serum cholesterol and phospholipid were also elevated, though to a lesser degree.

That there need not be a direct relation between the hepatic and serum lipids was illustrated earlier. In alcoholic cirrhosis the liver contains much fat, the serum usually little; in von Gierckе's disease, neutral fat is greatly increased in both. The opposite is found in this case of idiopathic hyperlipemia. Apparently, a high concentration of neutral fat in the serum does not necessarily provoke deposition of fat in the liver.

We believe that these observations lend credence to the aforementioned hypothesis that there is a mechanism which controls fatty infiltration of the liver.

TABLE 7.—*Liver Lipids in the Lipidoses (Diseases of Cellular Lipid Metabolism)*

	Normal Range, Gm. in 100 Gm.	Gaucher's Disease, Gm. in 100 Gm.	Niemann-Pick Disease, Gm. in 100 Gm.	Tay-Sachs Disease, Gm. in 100 Gm.	Essential Xanthomatosis, Gm. in 100 Gm.*
Total cholesterol.....	2.1 - 2.6	2.82	7.00	3.78	7.25
Free cholesterol.....	0.44- 0.55	0.14	4.50	0.47	2.70
Ester cholesterol...	1.50- 2.15	2.68	2.50	3.31	4.55
Total phospholipids	9.0 -11.0	8.90	37.10	9.26	7.40
Sphingomyelin	0.3 - 0.5	.....	25.9	0.55	0.19
Cephalin	3.0 - 5.5	0.84	11.20	Traces	5.21
Lecithin	3.0 - 6.0	.....	.....	8.71	2.00
Total fatty acids.....	8.6 -13.0	18.60	.....	27.40	9.05
Neutral fat	1.4 - 4.0	11.0	.....	19.4	0.71
Cerebrosides	.....	5.9	.....	.....	.....

*The liver analyzed was from a patient with generalized xanthoma of the normocholesteremic type.

Included in table 7 are the results of analyses of livers involved in three different diseases which according to Thannhauser and Magendantz [10] are due to intracellular disturbances of lipid metabolism. An outstanding feature is that in each disease only one definite lipid is involved. In Niemann-Pick disease it is sphingomyelin; in Gaucher's disease, cerebrosides, and in essential xanthomatosis there are increases of cholesterol, particularly of its esters. Besides these characteristic lipids, which are contained within the reticulum cells and histiocytes, a slight increase of neutral fat is sometimes found on chemical analysis. This increase in fat is usually of minor degree and bears no etiologic relation to the intracellular metabolic disturbance, being due perhaps to the general cachexia of these patients.

This type of fatty liver differs from others with regard to its cellular as well as its chemical composition. Histologically, numerous large cells

10. Thannhauser, S. J., and Magendantz, H.: Ann. Int. Med. **11**:1662, 1938.

are present which, according to their contents, are termed Gaucher cells, Pick cells or foam cells, the latter containing mostly cholesterol.

Included among the analysis relating to this group of intracellular lipid diseases is an analysis of liver in a case of Tay-Sachs disease. This condition was formerly believed to be closely related to Niemann-Pick disease. Chemical analysis, however, failed to reveal an increase of sphingomyelin or of any other phospholipid. The only demonstrable alteration manifested itself in the neutral fat fraction.

SUMMARY

Fatty livers obtained in cases of infectious and cachectic diseases, chronic alcoholic cirrhosis, toxemia of pregnancy, celiac disease, von Gierckе's disease, idiopathic hyperlipemia and the lipidoses, including essential xanthomatosis, Gaucher's disease, Niemann-Pick and Tay-Sachs diseases, were subjected to chemical analysis. Whenever possible, all lipid substances were determined.

It is emphasized that dry rather than wet tissue should be employed for lipid analyses. The obviation of technical difficulties by the use of the former tissue insures more uniform and comparable results.

It is believed to be possible by analytic partition of the lipids to differentiate between fatty infiltration and fatty degeneration. In cases of the former, values for hepatic neutral fat were found to be elevated and, although cholesterol and phospholipids were relatively low, their mutual proportions were maintained. In cases of the latter condition, however, the increase of neutral fat is less marked, the chief change being an alteration of the proportion between the cholesterol and other lipids. For the most part, simple fatty degeneration of the liver is quite uncommon (toxemia of pregnancy). More frequently, one encounters fatty livers whose pathologic alteration involves both fatty infiltration and degeneration. In cases in which one is more prominent than the other, chemical examination should often enable differentiation.

The suggestion has been made by other investigators [8] that a fat regulatory mechanism exists (perhaps hormonal) which protects against fatty infiltration of the liver. The observation that certain types of hyperlipemia occur without consequent infiltration of the liver, in our opinion, lends support to this hypothesis.

Liver obtained in a case of Tay-Sachs disease was examined. Formerly believed to be related to Niemann-Pick disease, this condition was shown on analysis to have no relation to a metabolic disturbance of the lipids. The simple fatty infiltration found was similar to that occasionally encountered in the various types of general cachexia.

OCCURRENCE OF ATHEROMA IN THE AORTA IN RABBITS WITH RENAL HYPERTENSION

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The cause of atheromatous plaques in the aorta has been the subject of much controversy, and many theories have been advanced in support of various agents.[1] Several of these theories have been emphasized repeatedly and have been tested either by experimental production of plaques or by prolonged clinical observation of patients with these lesions.

Cholesterol feeding of animals has been repeatedly shown to produce lesions which histologically and grossly are quite like those occurring spontaneously in man,[1b, c] and which many believe have the same fundamental etiologic factors as those of man,[2] although there are some dissenting opinions.[3]

High protein diets have been observed to produce atheromatous plaques in the aortas of rabbits,[4] although in these animals hypertension and renal lesions were also present.

The production of atheroma was noted in rabbits which had been hanged head down for a certain time daily over several months,[5] but these results could not be repeated.[6]

In man the diseases associated with excessive plaque formation in the aorta have been hypertension, diabetes and rheumatic fever. The

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1. (*a*) Crell, J. F., cited by Long, E. R., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 1. (*b*) Ignatowski, A.: Virchows Arch. f. path. Anat. **198**:248, 1909. (*c*) Anitschkow, N., cited by Long, E. R., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 10. (*d*) Osler, W.: Diseases of the Arteries, in Modern Medicine, ed. 2, Philadelphia, Lea & Febiger, 1915, vol. 4.

2. Leary, T.: Arch. Path. **17**:453, 1934. Rosenthal, S. R.: ibid. **18**:473 and 660, 1934.

3. (*a*) Duff, G. L.: Arch. Path. **20**:81, 1935. (*b*) Weiss, S., and Minot, G. R.: Nutrition in Relationship to Arteriosclerosis, New York, The Macmillan Company, 1933.

4. Clarkson, S., and Newburgh, L. N.: (*a*) J. Exper. Med. **43**:595, 1926; (*b*) Arch. Int. Med. **31**:653, 1923.

5. Klotz, O.: Centralbl. f. allg. Path. u. path. Anat. **19**:535, 1908.

6. Fahr, T.: Beitr. z. path. Anat. u. z. allg. Path. **15**:234, 1912.

most consistent finding associated with plaque formation, however, is senility, and it has been shown repeatedly that the degree of arteriosclerosis is directly proportional to the age of the patient, all other factors remaining constant.[8b]

In routine autopsies on rabbits rendered hypertensive by aortic constriction proximal to the origin of the renal arteries, it was found that the aorta in a considerable number of the cases showed small intimal plaques which were yellow and elevated and which microscopically had accumulations of fat-containing material.[7]

We were unable to find an explanation for these plaques by any of the known methods for producing arterial lesions. The experiments with these animals are reported in support of the view that this type of plaque formation is associated with hypertension.

MATERIALS AND METHODS

Twenty-seven adult rabbits weighing more than 2 Kg. each were used. Twelve were less than 1 year of age, but their exact ages were not known. The remaining 15 were known to be from 6 to 7 months of age. All of the latter were chinchilla rabbits of the same lineage and had been raised similarly from birth. The original 12 animals were of different types, the greater part being Belgian Hares and Flemish Giants.

All the animals were kept on stock diets of known cholesterol and protein content (protein 17 per cent and cholesterol 0.2 per cent), and the weekly intake for each animal was estimated at intervals during the experiment. Green material and carrots were given twice weekly, and water was supplied ad libitum.

In 18 of the animals aortic constriction was produced proximal and distal to the origin of the renal arteries to varying degrees, and in 9 of the animals the aorta was constricted to the same degree only distal to the origin of these arteries. All operative procedures were done with the animals under open drop ether anesthesia and with aseptic technic. Silver wire loops were used as the constricting medium.[6]

Determinations of blood pressure and examinations of the urine were made frequently during the first two weeks and at bimonthly intervals after that. Urine was tested for albumin and sediment at monthly intervals, and blood was taken for nonprotein nitrogen and cholesterol determinations at monthly intervals.

Ten of the animals were allowed to become pregnant; half of these had two or more pregnancies. It is noticeable that the number of pregnancies is directly correlated with the length of the life of the animal.

The animals were observed for varying periods following the constriction of their aortas, and those which had not died were killed at specified intervals by intravenous injection of 40 per cent solution of formaldehyde. Complete autopsies were made in all cases. Duplicate blocks were taken in Helly's modification of Zenker's solution[8] and in 10 per cent solution of formaldehyde. Routine brain

7. Dill, L. V.; Isenhour, C. E.; Cadden, J. F., and Kuder, A.: Surg., Gynec. & Obst. **72**:38, 1941. Dill, L. V.; Isenhour, C. E., and Cadden, J. F.: J. Clin. Investigation **18**:641, 1939.

8. Helly's modification is Zenker's solution prepared with neutral solution of formaldehyde U. S. P. instead of acetic acid.

sections were made and placed in 10 per cent solution of formaldehyde. All sections were stained routinely in hematoxylin and eosin, and fat stains were made by staining frozen sections with Harris' hematoxylin and scarlet red. Elastic tissue was stained by Weigert's elastic tissue stain and counterstained with trinitrophenol.

RESULTS

The clinical course and the observations at autopsy of animals with chronic hypertension produced by constriction of the aorta proximal to the origin of the renal arteries have been reported elsewhere.[7]

In the present study, 18 animals had constriction of the aorta proximal to the origin of the renal arteries. Of these animals, 15 had systolic pressure constantly elevated above 140 mm. of mercury. In 7 of these 15 animals atheromatous plaques developed. Three animals failed

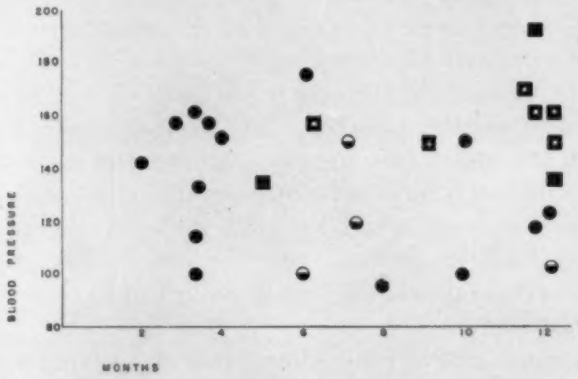

Fig. 1.—The distribution of animals in the experiment with regard to the duration and the degree of hypertension sustained. The hollow squares represent rabbits pregnant with atheroma formation; the hollow circles, rabbits pregnant without atheroma formation; the solid black squares, rabbits nonpregnant with atheroma formation, and the solid black circles, rabbits nonpregnant without atheroma formation.

to show hypertension to this degree, although the aorta was similarly constricted. In only 1 of these did aortic plaques develop.

Nine animals had the aorta constricted distal to the origin of the renal arteries. None of these had constant elevation of systolic blood pressure to 140 mm. of mercury. However, 2 animals did show constant readings around 135 mm. of mercury, and 1 of these showed two small plaques in the aorta.

The effect of pregnancy is demonstrated in figure 1. It is seen that 7 of the 15 hypertensive animals had sustained one or more pregnancies and that in 6 of the 7 atheroma had developed. Eight of the hyper-

tensive animals had never been pregnant, and in only 1 of these were plaques observed.

Of the 12 nonhypertensive animals, 3 had had pregnancies. Plaques were found in only 2 animals of the nonhypertensive group, and these had never been pregnant.

It is noticeable that no plaques occurred distal to the constricting clamp on the aorta and that none were seen on vessels of the systemic circulation or on the pulmonary artery.

The plaques occurred for the most part in the arch of the aorta and in the thoracic region (fig. 2 *A*). In many instances they were situated around the ostiums of the intercostal arteries. They were yellowish and elevated. The plaques ranged from 1 to 2 mm. in length and might be stretched out in streaks as long as 1 cm. They seemed to be entirely intimal. No medial injury was noted, but in 2 of our animals a medial lesion occurred that was not associated with the intimal type of change and was believed to have been spontaneous medial arteriosclerosis. No degeneration or calcification of the site of the lesion has ever been noted, and no ulceration has been seen.

Microscopically, the plaques merged gradually with the intima and seemed to be composed of an amorphous material containing a few nuclei and numerous cholesterol clefts. No proliferation of the endothelium was noted (fig. 2 *B*).

Elastic tissue stains showed the internal elastic lamella to be fibrillated and reduplicated (fig. 2 *C*).

Fat stains of this type of lesion showed that the amorphous material was largely composed of neutral fats and that most of this seemed to be in phagocytes. Doubly refractile bodies were also found with the polarizing microscope (fig. 2 *D*).

It would seem likely that the plaques observed in the aortas of these animals were not spontaneous in origin because of the high incidence of 33 per cent, compared with that of 1 to 2 per cent [9] for spontaneous intimal plaque formation in rabbits, and likewise because of the low incidence among the control animals of this series.

The effect played by diet, with regard to cholesterol and protein content, was not carefully controlled in the first group of animals, and it is in these animals that most of the plaques occurred. However, the stock diet was practically the same as that given to the last series except that no record was kept of the quantity taken and of how much supplementary green food was given. Blood cholesterol was determined for

9. Nuzum, F. R.; Elliot, A. H.; Richard, D. E., and Priest, B. O.: Arch. Path. **10**:697, 1930.

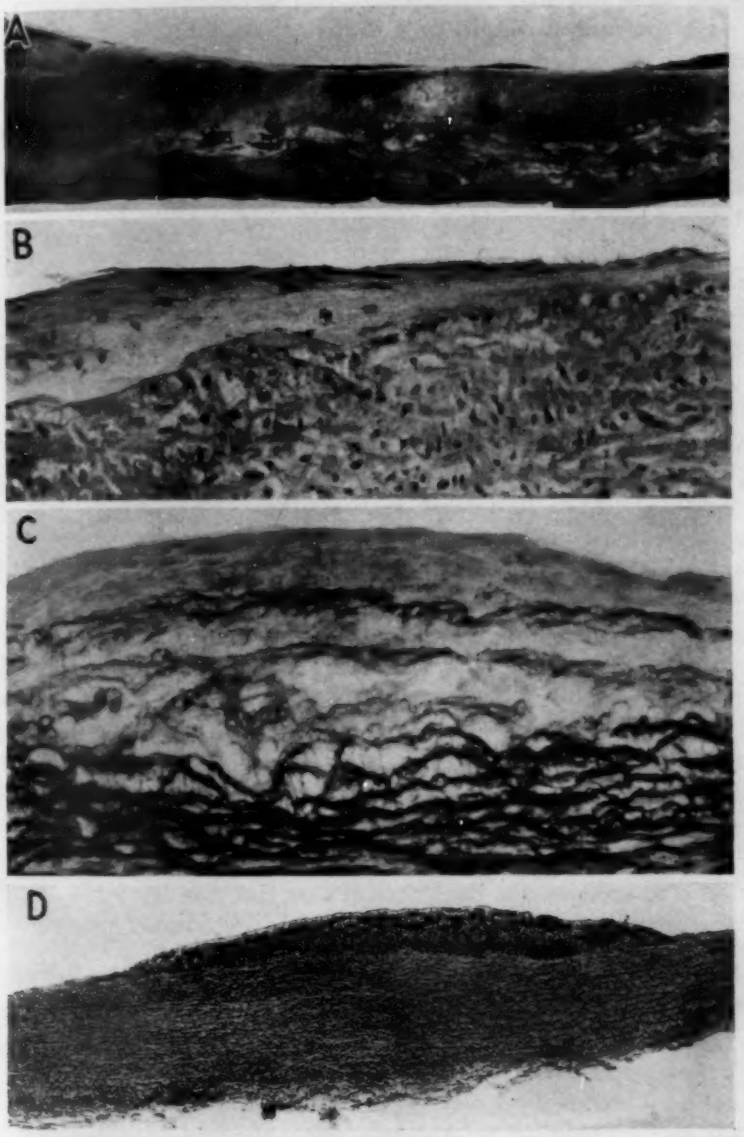

Fig. 2.—*A*, distribution of plaques in the thoracic aorta. *B*, longitudinal section through a typical plaque (hematoxylin and eosin). *C*, longitudinal section through a plaque showing fibrillation and reduplication of the internal elastic lamella (Weigert's elastic tissue stain). *D*, longitudinal section through a typical plaque showing a collection of free fat (scarlet red).

all animals, however, and no elevation was ever noted. The protein content was well below that quoted by Clarkson and Newburgh [4b] in the production of this type of lesion.

If a spontaneous origin or a dietary cause for this abnormality is ruled out, it seems logical to place emphasis on the elevation of blood pressure sustained by the aorta. We have found that the incidence of the plaques seems closely associated with the level of systemic pressure, that no lesions occur below the point of constriction in the aorta and that none occur in animals which have the same constricting band on the aorta but do not have elevated blood pressure levels.

If these lesions are related to the elevated pressure, we can only speculate as to the mechanism by which they occur. Winternitz and co-workers [10] have shown that spontaneous arteriosclerosis is closely associated with disruption of the blood supply to the subintimal areas by injury to the vasa vasorum which supply this region. On this same assumption it would seem likely that an increase of intra-aortic pressure if transmitted to any noticeable depth through the wall would interfere with filling of these vessels and cause anemia of the subintimal layer, such as Winternitz postulated as occurring. This process would, of course, be aided in those animals and patients in which a vasospastic condition is believed to exist, with consequent reduction of blood flow through this tissue (if all tissue can be represented by the kidney, on which flow studies have been made).

SUMMARY

Atheromatous plaques have been found in the aortas of rabbits which have had persistent elevation of blood pressure over long periods, produced by constriction of the aorta proximal to the origin of the renal arteries.

The plaque formation seems to be proportional to the severity of the elevation of pressure and the length of time that this acts; it is also seen more frequently in animals in which frequent pregnancies have occurred.

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10. Winternitz, M. D.; Thomas, R. M., and Lecompte, P. M.: Biology of Arteriosclerosis, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

SPONTANEOUS SOLITARY AND MULTIPLE MAST CELL TUMORS ("MASTOCYTOMA") IN DOGS

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Neoplasms of the skin and its accessory structures occur with great frequency in dogs. In my experience, they are the most common primary tumors in this species and their incidence greatly exceeds that of tumors occurring internally and that of neoplasms of the mammary glands. In a group of 49 primary tumors of the skin (excluding papillomas and circumanal gland tumors), I have encountered 5 (10.21 per cent) tumors whose histologic structure consisted essentially of somewhat atypical tissue or histogenous mast cells whose cytoplasm contained numerous metachromatic basophilic granules. Three of the tumors were solitary and benign and 2 were multiple and apparently malignant. Particularly in the latter group, numerous mast cells contained four distinct types of intracytoplasmic bodies.

With only 4 possible exceptions, no neoplasms of a similar type have been reported in the available human and veterinary medical literature. Sabrazès and Lafon [1] described on orange-sized tumor in the injured upper lip of a horse that consisted of eosinophils and mast cells. Fabris [2] noted multiple mast cell nodules, which he termed mastocytoma, in the subepithelial tissue of the skin in mice which had been intermittently exposed to finely pulverized tar in an enclosed atmosphere for several months, and Schreus [3] observed a nodule composed entirely of mast cells in the skin of a white mouse which had been painted with a neutral tar oil for three and a half months. In a report on a series of 4,000 experimental oil and tar tumors of the skin of mice, Twort and Twort [3a] mentioned tumors composed of mast cells which appeared to be benign, although a diffuse infiltration of the internal organs by mast cells seemed to have been the cause of the death of 1 of the animals.

The cytologic characteristics, tinctorial reactions and general distribution and arrangement of the neoplastic mast cells varied only slightly in the different tumors and for brevity will be described together. Differences existed between the solitary and the multiple tumors, and these will therefore be considered separately. The pertinent clinical data in

1. Sabrazès, J., and Lafon, C.: Folia haemat. **6**:3, 1908.
2. Fabris, A.: Pathologica **19**:157, 1927.
3. Schreus, H. T.: Dermat. Ztschr. **40**:9, 1924.
3a. Twort, C. C., and Twort, J. M.: Lancet **1**:1331, 1930.

each case will be briefly mentioned. The following terms will be applied to the mast cells comprising the tumor nodules: "neoplastic (or tumor) mast cells" and "neoplastic (or tumor) cells."

MATERIAL AND METHODS

The dogs whose cases are reported were animals brought to my animal hospital for treatment. The tissues were fixed in Zenker formaldehyde solution [4] and 10 per cent solution of neutral formaldehyde U. S. P. The sections were stained with hematoxylin and eosin, iron-hematoxylin, Mallory's phosphotungstic acid-hematoxylin, Mallory's aniline blue, Masson's trichrome stain,[4a] Van Gieson's stain, Unna's acid orcein, Wilder's reticulum stain, Unna's polychrome methylene blue, Dominici stain, alcoholic thionine, the May-Grünwald-Giemsa stain, the Unna-Pappenheim pyronine and methyl green stain and by Ellerman's modification of the May-Grünwald stain. In 1 case (4) imprints of the skin tumors, spleen, peripenile lymph nodes and bone marrow were stained with May-Grünwald-Giemsa.

SOLITARY TUMORS

Macroscopic Anatomy.—Case 1.—A 15 year old male brindle and white Boston terrier was brought to the hospital for destruction. The growth (fig. 1 *F*), which was asymptomatic and of unknown duration, was located in the right posterior femoral region. It was subepithelial and covered with normal epithelium. The nodule was elevated 10 mm. above the surrounding skin, which appeared normal and was 24 mm. in diameter. The tumor was firm, spherical, fixed to the overlying skin and uniform light gray and showed no capsule or trabeculae on section.

Case 2.—A 5 year old male tricolored English setter had for several weeks shown a growth in the right upper lip. The nodule measured 8 by 14 mm. and was embedded in the substance of the lip. The outline of the tumor could be observed extending above the skin externally and below the mucosa internally. The elevated surfaces were rounded, and the skin and the mucosa appeared normal. The tumor was firm and of a uniform grayish white color. A capsule and trabeculae were absent. A section was taken for histologic study, and the neoplasm was removed by surgical diathermy on Nov. 15, 1940. There has been no recurrence to the present.

Case 3.—An 8 year old female tricolored wire-haired fox terrier had a nodule of several months' duration located on the medial surface of the left external auditory meatus. The animal scratched at it constantly, so that it bled. The tumor was oval and elevated 14 mm. above the surrounding skin, which appeared normal. It was 18 mm. in diameter, and the epithelium was ulcerated in areas. The outer circumference was a brownish gray color, and the central portion of the nodule, which appeared soft and friable, was a deep reddish brown color. The tumor was excised on July 31, 1940, and there has been no recurrence to date.

Microscopic Anatomy.—(*a*) Cellular Detail: In general, the tinctorial properties and cytologic aspects of the neoplastic cells in the three tumors were similar. The majority of the cells were round, oval or polyhedral, although elongated, spindle-shaped and piriform types occurred. The tumor cells measured from 5

4. This is Zenker's solution prepared with neutral solution of formaldehyde U. S. P. instead of glacial acetic acid.

4a. Masson, P.: J. Tech. Methods **12**:75, 1929.

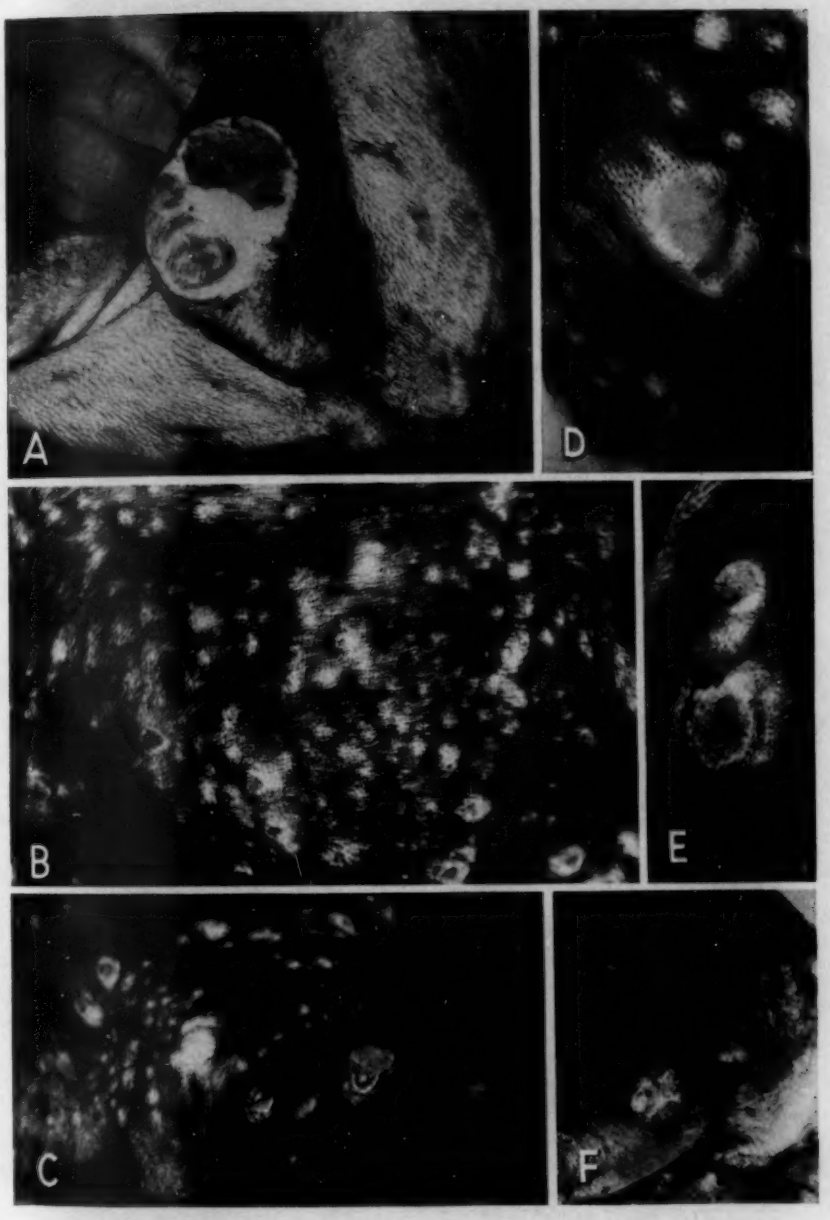

Fig. 1.—*A*, gross appearance of scrotal nodule in case 4. *B*, *C*, *D* and *E*, gross appearance and distribution of multiple cutaneous nodules in case 4. *F*, gross appearance of solitary nodule in case 1.

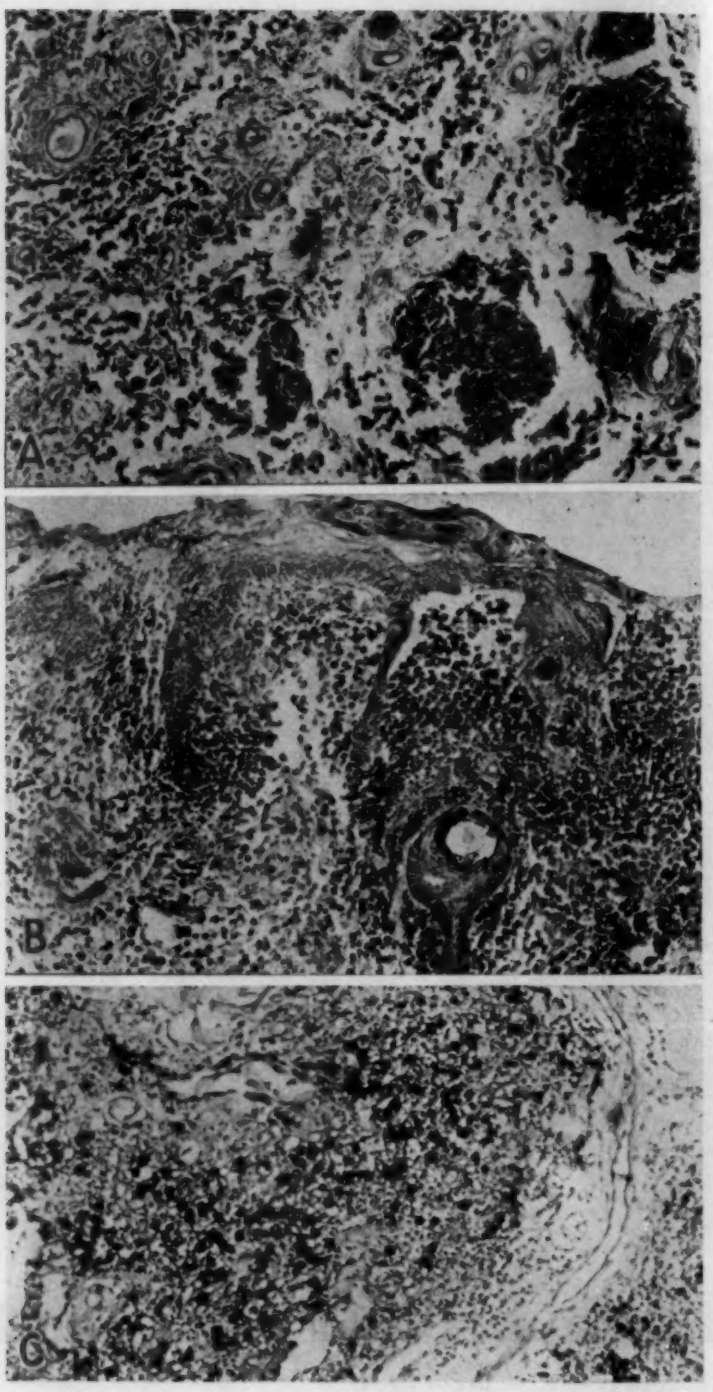

Figure 2

(See legend on opposite page)

to 18 microns in diameter, with an average size of 9.9 microns, in paraffin sections. They exhibited no polymorphism, although occasional cells possessed granule-containing cytoplasmic prolongations.

The nuclei ranged from 4 to 9 microns in length, with an average of 5.5 microns. They were usually round or oval, occasionally elongated or indented and never polymorphous. The nucleus was relatively vesicular, although there was a well defined, somewhat reticular, irregular, thin chromatin network that was often aggregated in small coarser clumps. These were frequently marginated on a distinct nuclear membrane. Irregularly enmeshed in the nucleoplasm were one and occasionally two large spherical acidophilic nucleoli. A moderate number of cells were binucleated, and mitotic figures were absent.

The cytoplasm was characterized by the presence of numerous metachromatic basophilic granules, which were usually spherical and occasionally oval. In some cells the granules were so plentiful that the nucleus was obscured. In other cells there was a clear perinuclear zone, free from granules. Fine and coarser granules were present in the cytoplasm of the same cell, and occasionally the granules appeared fused to form large, coarse, spherical, deeper staining granular masses. In a few cells the cytoplasm was vacuolated, distinct granules were absent, but a threadlike metachromatic basophilic material was present. With occasional cells, there was a scattering of the mast granules outside of the cell bodies.

The tinctorial properties of the granules and other cellular constituents were as follows: With hematoxylin and eosin, the nucleus was blue, the nucleolus pink and the cytoplasm red and in some cells the granules stained blue; with Van Gieson's stain, the cytoplasm was yellow and the granules unstained; with Masson's trichrome stain, the granules were unstained and the cytoplasm was green; with Mallory's aniline blue, the cytoplasm was bluish, and in many cells the granules were stained a deep blue; with iron-hematoxylin, Mallory's phosphotungstic acid–hematoxylin and Wilder's reticulum stain, the granules were unstained; with Unna's polychrome methylene blue, the granules were reddish, and all other cellular constituents were blue; with Dominici's stain, alcoholic thionine, the May-Grünwald-Giemsa stain and Ellerman's modification of the May-Grünwald stain, the granules were reddish purple, the nuclei blue and the nucleoli pink; with the Unna-Pappenheim pyronine and methyl green stain, the nucleus was blue-green and the granules deep reddish purple.

The intracytoplasmic bodies, of which a detailed description will be given under the heading of multiple tumors, occurred in the solitary neoplasms only occasionally.

(*b*) Neoplastic Structure: In 2 cases (1 and 2) the epithelium was flattened, distinct papillae were absent and degenerative epithelial changes were lacking. In case 3 the epithelium was disorganized and ulcerated in areas and the interpapillary processes were lengthened and sometimes thickened (fig. 2*B*). In all the cases the cutis was edematous in areas and some lymphatic vessels were dilated.

EXPLANATION OF FIGURE 2

A, solitary nodule with diffuse and nodular collections of neoplastic mast cells (case 1). Hematoxylin and eosin; × 70.

B, solitary nodule showing epithelial changes, with lengthening of interpapillary processes and heavy neoplastic mast cell distribution (case 3). Dominici stain; × 70.

C, solitary nodule illustrating irregular distribution of tumor mast cells in the corium (case 2). The smaller cells are eosinophils, of which large numbers occurred in this nodule. Unna's polychrome methylene blue; × 70.

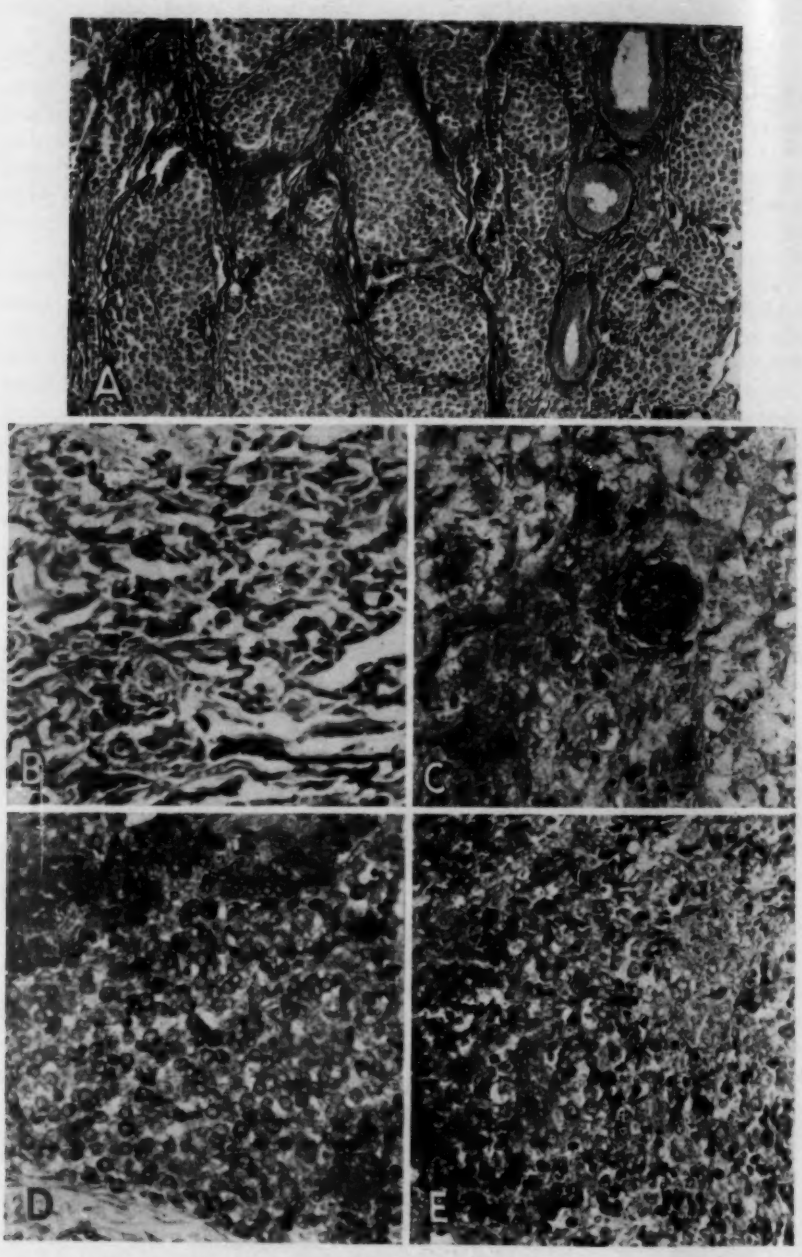

Figure 3

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The corium and subcutaneous tissue were heavily infiltrated with numerous neoplastic mast cells (fig. 2*A*, *B* and *C*). Many times the tumor cells were heavily grouped into smaller and larger nodular collections (fig. 2*A*). In other regions the cells loosely infiltrated the connective tissue and muscle bundles. There was no definite distributional pattern of the invading cells as far as blood vessels and glands were concerned, and in the areas of heavy infiltration, hair follicles, apocrine and sebaceous glands were usually absent. The pars papillaris in the cases with intact epithelium was relatively free from tumor cells. There was no connective tissue proliferation to form trabeculae or a capsule. Scattered among the tumor cells were variable numbers of eosinophils with occasional neutrophilic polymorphonuclear leukocytes, plasma cells, lymphocytes and histiocytes. There were no intra-epidermal tumor mast cells. The epithelium and corium adjacent to the infiltrated region appeared normal. At the immediate junction of the tumor and the normal tissues a moderate number of neoplastic mast cells infiltrated the otherwise unchanged cutis.

Inflammatory reactions were absent with the exception of case 3. The central portion of the nodule was necrotic with many thin-walled blood vessels, hemorrhage and polymorphonuclear leukocytic infiltrations. In this region were numerous degenerated neoplastic mast cells with poorly staining or pyknotic nuclei and a variety of granule changes. Many times these stained faintly; occasionally they were clumped and formed coarse, irregular granular masses that stained in an aberrant tone; the cytoplasm was often vacuolated and contained irregular strands that frequently retained a metachromatic tint. A moderate number of intact tumor cells were present with well preserved nuclei and distinct metachromatic granules. There was marked scattering of granules throughout the necrotic area. In this region, the connective tissue, reticulum and elastic fibers were scanty and ill defined. In the intact nodules, the fibrous connective tissue was decreased in the areas of heavy cellular grouping, although fine argyrophilic fibers were usually present. In the areas of relatively lighter cellular infiltration, the collagenous fibers appeared normal.

EXPLANATION OF FIGURE 3

A, cutaneous nodule showing irregular grouping of closely packed neoplastic mast cells with relatively avascular connective tissue stroma (case 4). Mallory's phosphotungstic acid–hematoxylin; × 67.

B, nodule of scrotal skin showing pleomorphism of tumor mast cells (case 4). The cellular arrangement and fibrous stroma are different from those in *A*. Dominici stain; × 223.5.

C, liver (case 4) showing slight periportal fibrosis and solitary and nodular tumor mast cell collections in the periportal tissue and in the sinusoid in the right upper corner of the figure. The hepatic cells show moderate degenerative changes. Unna's polychrome methylene blue; × 223.5.

D, peripenile lymph node (case 4) illustrating diffuse infiltration of tumor mast cells between a medullary cord above and a thickened trabecula below. Unna's polychrome methylene blue; × 223.5.

E, spleen (case 4) showing infiltration of neoplastic cells in the red pulp. The extremely deep-staining cells are normoblasts and plasma cells. Unna's polychrome methylene blue; × 223.5.

MULTIPLE TUMORS

Macroscopic Anatomy and Clinical Data.—Case 4.—A 9 year old male black spaniel cross was brought to the hospital on May 4, 1940 with a history of pruritis of the scrotum of several weeks' duration. On examination the scrotal skin was found involved in an oval hairless nodule which was elevated, with a reddened, flat surface (fig. 1 *A*). The lesion measured 23 by 39 mm. and was firm in consistency. In addition, there were several small spherical palpable nodules in the subcutaneous scrotal skin. The peripenile lymph nodes were enlarged. The testes appeared normal. With the animal under local anesthesia, the entire scrotum and testes were removed. The animal was discharged from the hospital on May 13, with complete healing of the operative site. On June 18 the animal was brought back with a history of intense generalized pruritus of one week's duration. The dog was clipped, and the entire integument was literally covered with skin tumors (fig. 1 *B*). These were most numerous on the back and head regions. Urinalysis revealed no abnormalities. Blood examination showed: red cells 3,590,000, white cells 60,600 and hemoglobin (Newcomer's method) 8.83 Gm. per hundred cubic centimeters. The differential percentages were: segmented cells 55.75, stab cells 38.25, juvenile cells 0.5, monocytes 1.5, lymphocytes 3.5, basophils 0.25 and eosinophils 0.25. The platelets appeared normal, and there were 28 nucleated red cells per 400 white cells counted. Numerous polychromatophils were present, and there was moderate anisocytosis with many macrocytes. The owner requested destruction, which was accomplished with a lethal dose of soluble pentobarbital given intravenously.

Necropsy revealed no gross anatomic changes with the exception of the skin tumors and the enlarged peripenile lymph nodes. No nodules were present at the previous operative site.

The skin nodules (fig. 1 *B* to *E*) were subepithelial and varied from 6 to 36 mm. in diameter. They were round or oval and closely adherent to the overlying epithelium but not to the underlying subcutaneous tissue. They were elevated 6 to 10 mm. above the surrounding skin, which appeared normal. In some nodules there was superficial ulceration of the epithelium. The surface of the tumors were flattened, sometimes rounded and occasionally umbilicated. They were firm and uniformly pale fleshy tan in color. Trabeculae and capsule were absent on section.

The peripenile lymph nodes measured 13 by 35 mm., were moderately firm, nonadherent to the surrounding tissues and pale brown.

Case 5.—The owner of a 15 year old male brown and white fox terrier observed nodule formations on its scrotum for several months. The animal was treated by another veterinarian, who removed several large nodules on Aug. 7, 1940. The operative site became infected and necrotic, and the animal was brought to me for consultation on August 12. In addition to scrotal nodules similar to those which were observed in the previous case, the scrotal skin was thickened, ulcerated and edematous. The peripenile lymph nodes were enlarged, and cutaneous tumors were absent. With the dog under local anesthesia, the scrotum, testes and regional lymph nodes were removed. The operative site healed nicely, but the animal died on August 29. Unfortunately, permission for necropsy examination was unobtainable.

Microscopic Anatomy.—(*a*) Cellular Detail: The neoplastic cells showed little variation from those in the solitary tumors (fig. 4 *D*). The staining reactions of the nuclei, cytoplasm and granules were the same except that the latter decolor-

ized more easily. In addition, many cells were binucleated, and occasional mitotic figures were present. In a moderate number of cells, two and even three nucleoli were irregularly disposed in the nucleus. In rare cells the nuclei were vacuolated. In paraffin sections the cells varied from 6.6 to 12 microns, with an average length of 8.8 microns. The nuclei measured from 4 to 8 microns and averaged 5.3 microns in size. In imprints of the skin, peripenile lymph nodes, spleen and bone marrow, the cells ranged from 8 to 22 microns in diameter, with an average of 14.2 microns. The nuclei were from 6.5 to 10.6 microns, with an average of 8.9 microns. Imprints from the different organs showed a uniform structure of the neoplastic cells (fig. 4 *A, B* and *C*). The great majority were spherical, with numerous deeply staining granules that frequently obliterated the nucleus. The cytoplasm was occasionally seen to be stained pale blue. The nucleus was round or oval, with a distinct chromatin pattern, and contained from one to three nucleoli. The cytoplasm was occasionally vacuolated, and there was frequent granule scattering.

The cytoplasm of numerous cells contained four different types of bodies that varied in appearance and in staining reactions; two or more frequently occurred simultaneously in the same cell. They were as follows:

1. An elongated, rod-shaped, thin crystalloid (about the thickness of an individual granule) of uniform thickness. It was almost constantly straight but occasionally curved, showed no internal structure and stained uniformly (fig. 4 *E*). The terminal portions were slightly rounded, and in occasional thicker crystalloids there was a paler staining central portion. These structures were haphazardly arranged in the cytoplasm, were about as long as the nucleus and often extended the entire length of the cell. In a moderate number of cells two such bodies were present, and occasional cells had three and even four. In these cells they were sometimes lined closely together in a parallel manner, were crossed or occupied opposite regions of the cytoplasm. They were often superimposed on the nucleus, and in many cells tangential and cross sections could be seen. These structures occurred only in the tumor cells and were absent in the interstitial tissue. The crystalloids were best differentiated with iron-hematoxylin, which stained them black and Mallory's phosphotungstic acid–hematoxylin, which stained them blue. They were often seen with Unna's polychrome methylene blue, with which they stained blue, and with Dominici's stain, which stained them purple-blue. They were difficult to detect with hematoxylin and eosin; when observed, they stained pale purple. They were not stained in imprints with May-Grünwald-Giemsa stain.

2. A spherical, deeply basophilic, uniformly stained body that was usually completely surrounded by an unstained granule-free cytoplasmic halo (fig. 4 *F, a*). These bodies were well stained with the majority of the methods used, but were particularly well brought out by iron-hematoxylin, Mallory's phosphotungstic acid–hematoxylin, Unna's polychrome methylene blue and the Unna-Pappenheim pyronine and methyl green, with which they were deeply basophilic. They varied in size from that of a nucleolus to that of a nucleus, and two or more occasionally occurred in the same cell. The nuclei of these cells were often indented and the bodies occupied the position of the *Hof*.

3. Similar spherical bodies of the same size and staining reactions with slightly irregular edges (fig. 4 *F, b*). Their internal structure appeared to consist of numerous closely packed granules, which in regions appeared to have fused and formed small solid masses. These were likewise surrounded by a clear cytoplasmic zone.

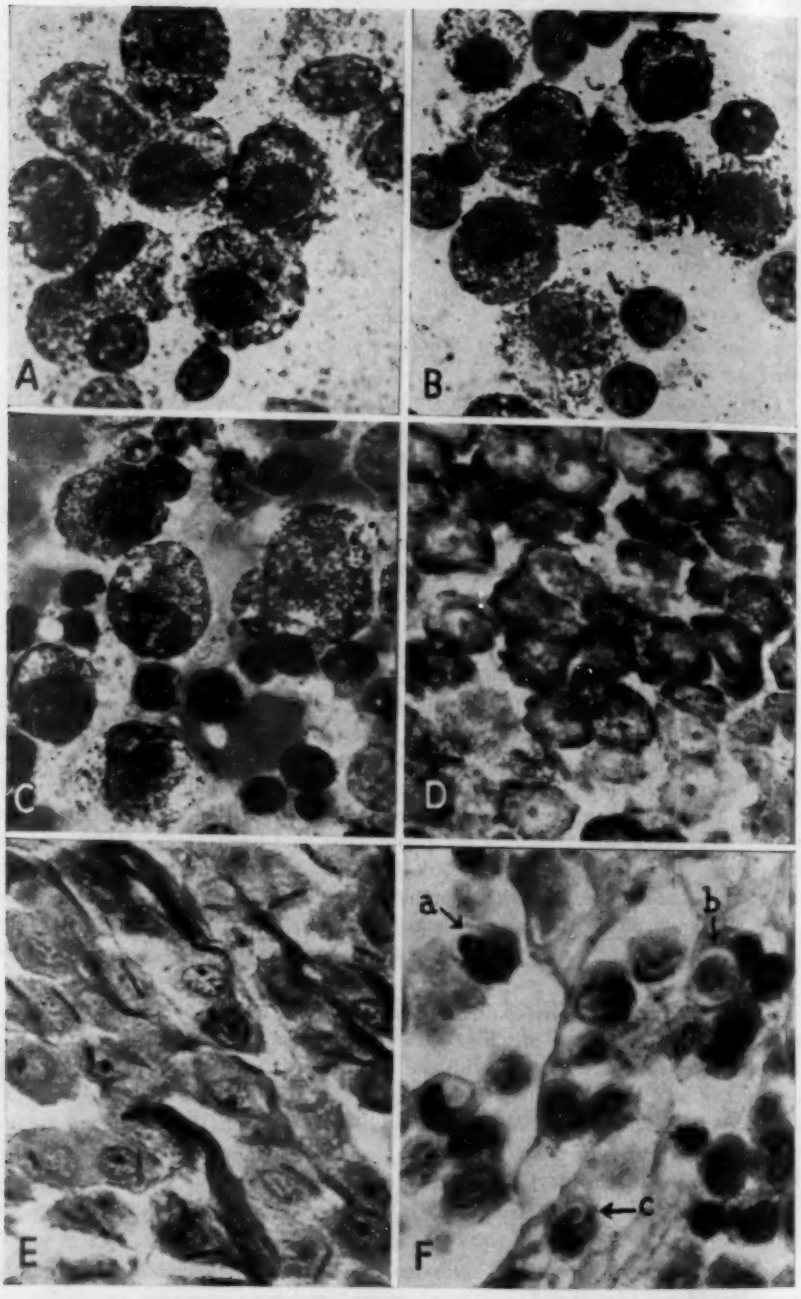

Figure 4

(See legend on opposite page)

4. Spherical bodies surrounded by unstained halos, which were acidophilic, often had a uniform internal structure and were occasionally granular (fig. 4 *F*, *c*).

(*b*) Skin: The neoplastic nodules of the skin and the scrotum were alike with slight differences. In some nodules the epithelium was necrotic and replaced by polymorphonuclear leukocytes, red cells and degenerated epithelial cells. Immediately below the ulcerated epithelium, the papillary layer was edematous and infiltrated with numerous polymorphonuclear leukocytes. In case 5 there were marked edema, hemorrhage and inflammatory changes throughout the epithelium and cutis. In other nodules the epithelium was normal. The entire corium and subcutis were heavily infiltrated with closely packed neoplastic mast cells, and hair follicles, apocrine and sebaceous glands were absent. These often occurred in irregular groups and masses separated by thin strands of relatively avascular fibrous connective tissue and argyrophilic fibers that only occasionally penetrated the cellular collections (fig. 3 *A*). In the scrotal skin the same general cellular arrangement was maintained, although large areas of the corium were free from tumor cells. In several scrotal nodules the cells showed marked pleomorphism (fig. 3 *B*). Scattered among the tumor cells were occasional lymphocytes, plasma cells, eosinophils, histiocytes and neutrophilic leukocytes. There were no intraepidermal neoplastic cells.

(*c*) Peripenile Lymph Nodes: Numerous tumor mast cells had invaded the capsule, trabeculae, sinuses and interfollicular tissue (fig. 3 *D*). Many hyperplastic follicles were present with prominent secondary nodules. The structural pattern was obscured, and the medullary cords were indistinct. The sinuses had almost lost their identity, so heavily were they infiltrated with neoplastic cells. Solitary tumor cells frequently invaded the outer follicular rim. Throughout the organ were many plasma cells, occasional eosinophils and macrophages containing hemosiderin. The capsule and trabeculae were thickened as a result of connective tissue proliferation. There was no necrosis, and inflammatory reactions were absent.

(*d*) Spleen: The general structural pattern was undisturbed. The follicles were normal, and many had secondary nodules. There were numerous mega-

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EXPLANATION OF FIGURE 4

A, imprint of cutaneous tumor (case 4) showing general structure of the neoplastic mast cells. There are granule scattering, vacuolation of the cytoplasm and in one cell a deeply staining basophilic spherical body. May-Grünwald-Giemsa stain; × 966.5.

B, imprint of a peripenile lymph node (case 4). Several lymphocytes are present in addition to the tumor mast cells. May-Grünwald-Giemsa stain; × 966.5.

C, imprint of spleen (case 4) showing numerous normoblasts in addition to neoplastic cells. May-Grünwald-Giemsa stain; × 966.5.

D, paraffin section of a nodule of the scrotal skin (case 5) showing numerous granules in the tumor cells. Dominici stain; × 966.5.

E, nodule of the scrotal skin (case 4) showing numerous crystalloids in the neoplastic mast cells. In some cells tangential and cross sections of the crystalloids can be seen. Mallory's phosphotungstic acid–hematoxylin; × 966.5.

F, cutaneous nodule (case 4) showing spherical, deeply basophilic solid body (*a*), spherical, basophilic granular body (*b*) and spherical, acidophilic body (*c*). Iron hematoxylin and eosin; × 966.5.

karyocytes and plasma cells and occasional eosinophils and neutrophilic leukocytes. Congestion was absent, and many foci of erythrogenesis were present. Throughout the pulp were numerous neoplastic mast cells that often formed small nodular collections (fig. 3 *E*). Solitary tumor cells were diffusely distributed through the tissue and frequently invaded the malpighian corpuscles.

(*e*) Liver: The liver cells showed generalized moderate parenchymatous degeneration. There was no congestion, and occasional megakaryocytes were present in the sinusoids. As evidence of hepatic regenerative activity there were binucleated cells, mitotic figures and hyperchromatic nuclei. The liver cords were normal, and some Kupffer cells contained hemosiderin and fragmented red cells. There was slight periportal fibrosis, and the periportal tissue was infiltrated with plasma cells and solitary and nodular collections of neoplastic mast cells (fig. 3 *C*). These cells were easily differentiated morphologically from the tissue mast cells which occur in relatively large numbers in the liver of the dog.[5]

(*f*) Adrenal: The adrenal showed no changes and contained no neoplastic mast cells. There were solitary and nodular collections of neoplastic cells in the periadrenal tissue.

(*g*) Omentum and Mesentery: Numerous solitary and nodular collections of neoplastic mast cells were present.

(*h*) Lung: Throughout the interalveolar tissue were numerous tumor mast cells which usually occurred singly or in small groups. Distinct nodular collections were not observed, and the lungs were normal.

(*i*) Bone Marrow: Paraffin sections of decalcified sternum showed hyperplastic marrow with numerous erythroid cells. There was a reversal of the myeloid-erythroid ratio as determined by cell counts of bone marrow imprints. Of 500 marrow cells counted, 2.2 per cent were neoplastic mast cells. Basophilic myelocytes and leukocytes were not present.

(*j*) Other Organs: Sections of other tissues and organs, including the lymph nodes, showed an absence of neoplastic mast cells. The usual tissue mast cells were present, and these showed no morphologic changes.

COMMENT

The presence of mast cells in the tissues of normal animals and man is well known. According to Michels,[6] they are numerically increased in the following cutaneous disorders: urticaria pigmentosa, urticaria urens, hypotrichosis, acne of the nose, iodine poisoning, vesications, scabies and eczema, syphilitic condyloma, hypertrophic angioma, subcutaneous lipoma, papilloma and xanthoma. In carcinomatous areas the tissue mast cells are usually absent, but they are increased in the adjacent normal tissue and the regional lymph nodes.[7] An abundance of mast cells has been noted in uterine fibromyoma, neurofibroma, angio-

5. Nakajima, Y.: Tr. Jap. Path. Soc. **18**:150, 1928.

6. Michels, N. A., in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 1, p. 232.

7. Fromme, F.: Zentralbl. f. Gynäk. **30**:1146, 1906. Weill, P.: Folia haemat. **23**:185, 1919. Staemmler, M.: Frankfurt. Ztschr. f. Path. **25**:391, 1921. Higuchi, K.: Folia haemat. **41**:401, 1930.

myoma, endothelioma, lipoma and in the subcutaneous tumors in von Recklinghausen's disease.[6] Bierich [8] and Fabris [2] observed a tremendous increase of the tissue mast cells in cutaneous tumors experimentally produced by the use of tar.

The causative factor for the increase of tissue mast cells in the lesions mentioned is unknown, although these cells undoubtedly arise secondarily, in response to the stimulus of the initial pathologic changes. The morphologic and distributional characteristics of the tissue mast cells show slight, if any, resemblance to the mast cell tumors. The granuloma described by Sabrazès and Lafon [1] and the mast cell nodules produced by Fabris [2] and Schreus [3] likewise varied in histologic features.

The question of diagnostic classification immediately arose, and several possibilities were considered. The nodules were not secondary to any primary neoplasm of the skin or other cutaneous lesion of a specific type. That they were granulomatous lesions can be discounted because of the absence of any proliferation of tissue resembling inflammatory granulation tissue. With the multiple tumors, thought was given to the probability of aleukemic mast cell leukemia. I [9] have observed basophilic myelocytic leukemia in several cats but in no dogs. The bone marrow imprints showed a quantitative increase in basophilic cells (2.2 per cent) as compared with the average normal of 0.13 per cent.[10] However, the mast cells were morphologically identical with those in the cutaneous nodules and other organs. It is probably justifiable to term these nodules tumors in the broader sense or perhaps even in the more narrow interpretation of the word. The name "mastocytoma" is therefore proposed for them. If the terms "histiocytoma" and "plasmacytoma" are acceptable, the designation "mastocytoma" should likewise be appropriate.

The genesis of the neoplastic mast cells offers several possibilities. They are apparently not of hemic origin; the lack of nuclear polymorphism, the larger size and the absence of the cells (including the multiple tumors) in the blood vascular system militate against their being basophilic leukocytes. They are not basophilic myelocytes that have been transported via the vascular system, and there is no indication that they have developed locally in an extramedullary manner. In addition, the basophilic myelocytes are smaller, have a relatively larger nucelus and and a comparatively narrow rim of cytoplasm. The evidence appears to indicate that they are of histogenous origin. In the normal adult organism the supply of tissue mast cells is maintained by homoplastic and heteroplastic regeneration.[6] The former is accomplished by mitotic division of preexisting tissue mast cells and the latter

8. Bierich, R.: Virchows Arch. f. path. Anat. **239**:1, 1922.
9. Bloom, F.: Unpublished data.
10. Alexandrov, A. F.: Folia haemat. **41**:428, 1930.

by elaboration of mast granules in various types of connective tissue cells, as lymphocytes, plasma cells, clasmatocytes, adventitial cells and histiocytes.[6] In no instances were there present intermediate cell types with mast-granulopoiesis or with nuclear and cytoplasmic characteristics of the various types of connective tissue cells that indicated a heteroplastic origin. The normally occurring tissue mast cells of the dog differ from the tumor cells in the following respects: The majority are elongated and spindle shaped, although oval and spherical forms occur; the nucleus is relatively smaller, and the cells are polymorphous. The granules are alike in appearance and in tinctorial reactions, and intracytoplasmic bodies are absent. It seems reasonable to assume that the neoplastic mast cells arose from proliferation of the preexisting tissue mast cells and in this process lost their polymorphism and acquired a generally more oval or spherical form and a larger, relatively vesicular nucleus. This transformation was accomplished in the solitary tumors by amitotic activity, as evidenced by binucleation and occasionally constricted, elongated nuclei, and in the multiple tumors by mitoses and amitoses.

The question of the malignancy of the multiple tumors is debatable. The primary tumors apparently originated in the scrotal tissue, and the rapid development of the numerous cutaneous tumors (case 4) suggests a multicentric origin. The presence of the neoplastic cells in the regional lymph nodes, the spleen, the liver and other organs may be evidence of metastasis or may also indicate a multicentric origin. In several scrotal nodules the tumor cells showed marked pleomorphism, which may be accepted as a criterion of malignancy. If the neoplastic cells developed multicentrically, an explanation must be found for the absence of these cells in tissues and organs in which tissue mast cells are normally present. It was extremely difficult to ascertain the presence of tumor cells in the lymphatic vessels of the cutaneous tumors, although they were readily observed in the sinuses and lymph vessels of the regional lymph nodes.

The intracytoplasmic bodies in the neoplastic mast cells are of exceptional interest and merit close attention. This is particularly true of the rod-shaped structures, which from their appearance and staining reactions are undoubtedly crystalloids. Somewhat similar bodies occur normally in the Sertoli cells, primary spermatogenic cells and interstitial cells of the testes of man (but not in those of dogs) that are either spindle shaped with pointed ends or rod shaped with rounded or pointed extremities.[11] Crystalline material of a possibly protein nature has been

11. Maximow, A. A., and Bloom, W.: A Text-Book of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1939, p. 490. Rasmussen, A. T., in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 1675.

described as occurring in multiple myeloma [12] and in reticuloendotheliosis.[13] The radial inclusions of the giant cells observed in lesions simulating tuberculosis or foreign body granulation tissues and which are present in a variety of pathologic conditions are believed to be crystalline forms of fat.[14] There is little resemblance between this crystalline material and the crystalloids in the tumor mast cells. The refringent inclusions noted by Broderson [15] in the tissue mast cells after implantation of hair, needles and carbon in the connective tissue also differed from the crystalloids. The available literature contains no references to identical crystalloids occurring in any cellular types, including the tissue mast cells. These structures are of a unique nature, and their composition, origin and function, if any, are unknown.

The spherical cytoplasmic bodies may represent substances of endogenous or exogenous origin. It is well known that tumor cells may have phagocytic properties and ingest a variety of substances, such as red cells, leukocytes, other tumor cells, bacteria, pigment and foreign bodies that may all be in stages of degeneration.[16] From the available literature,[6] it is doubtful that tissue mast cells are capable of extended phagocytosis, although this process has been reported and apparently substantiated. That these bodies are of endogenous origin appears more plausible. They may conceivably represent the products of degenerative changes of the cytoplasm and its constituent granules. Furthermore, the possibility that they may be virus inclusion bodies, while of distinctly conjectural nature, cannot be overlooked. It is doubtful if they are artefacts resulting from the use of aqueous fixatives and stains, because of the uniform structure and staining reactions. The spherical basophilic bodies resemble the bird's eye inclusions of Leyden, which occur in glandular carcinoma but are absent in sarcoma and epidermal carcinoma and may arise from multiplication of centrosomes or from secretory processes in which centrosomes are probably concerned.[16] Against this concept is the evidence that the neoplastic mast cells are of undoubted connective tissue origin, while the bird's eye inclusions are present in carcinomatous cells in which secretory function is present or may be assumed to persist. The microscopic appearance of the spherical bodies appears to indicate that they arise from fusion or agglutination of the granules. In this process the resulting spherical mass stains a

12. Glaus, A.: Virchows Arch. f. path. Anat. **223**:301, 1917. Abrikosoff, A., and Wulff-Moskau, F.: Verhandl. d. deutsch. path. Gesellsch. **22**:270, 1927.

13. Ritchie, G., and Meyer, O. O.: Arch. Path. **22**:729, 1936. Agress, H., and Smith, M. G.: ibid. **29**:553, 1940.

14. Hirsch, E. F.: Arch. Path. **20**:665, 1935.

15. Broderson, J.: Ztschr. f. mikr.-anat. Forsch. **14**:60, 1928.

16. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 31.

deep basophilic tone with loss of metachromasia of the individual granules. The spherical bodies with a granular structure that often retains metachromatic properties are undoubtedly intermediate stages in this process.

SUMMARY

The term "mastocytoma" is proposed for a hitherto undescribed primary subepithelial neoplasm occurring spontaneously in dogs. These tumors are of unknown cause and may be solitary and benign or multiple and apparently malignant. The histologic structure consists of somewhat atypical tissue mast cells, which are apparently of histogenous origin and arise from preexisting tissue mast cells by mitosis and amitosis. In addition to numerous metachromatic basophilic granules, the cytoplasm of the neoplastic mast cells contains rod-shaped crystalloids, spherical basophilic bodies of solid or granular structure and spherical acidophilic bodies. The intracytoplasmic structures occurred in numerous cells of the multiple tumors and in occasional cells of the solitary tumors.

ROLE OF PARASITE PIGMENT (FERRIHEMIC ACID) IN THE PRODUCTION OF LESIONS IN MALARIA

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The mechanism of tissue injury in malarial infections and in the complication of malaria known as blackwater fever is still undetermined. While general and local anemia [1] is in large part responsible for the symptoms and the pathologic changes, specific toxic factors also may be implicated. Thus, Taliaferro and Cannon [2] observed in monkeys with heavy malarial infections nephrosis with degeneration of convoluted tubular epithelium. This evidence and other considerations led them to suggest that some toxic material is liberated during such infections.

Malarial pigment, derived from hemoglobin and apparently an end product of the intracellular metabolism of the parasite, has been incriminated as a toxic factor in a series of papers by Brown.[3] While the pigment had been known widely as melanin, Brown described in detail the many differences between the two. It has now been demonstrated [4] that the malarial parasite pigment is ferrihemic acid (hematin).

We [5] have described elsewhere the pathologic changes induced in dogs by injected ferrihemate, which simulate lesions occurring in human malaria and blackwater fever. Since malaria is unknown in dogs we

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This study was aided by a grant from the Tennessee Valley Authority through the Department of Preventive Medicine of the University of Tennessee.

The strain of malaria parasites used in the experiments was supplied by Dr. L. T. Coggeshall, of the Laboratories of the International Health Division of the Rockefeller Foundation in the Rockefeller Institute for Medical Research.

1. Morrison, D. B., and Anderson, W. A. D.: On the Role of Parasite Pigment in the Malaria Paroxysm, Pub. Health Rep. **57**:161, 1942.

2. Taliaferro, W. H., and Cannon, P. R.: J. Infect. Dis. **59**:72, 1936.

3. Brown, W. H.: J. Exper. Med. **13**:290, 1911; **14**:612, 1911; **15**:579, 1912; **18**:96, 1913; Arch. Int. Med. **12**:315, 1913.

4. Morrison, D. B., and Anderson, W. A. D.: The Pigment of the Malaria Parasite, Pub. Health Rep. **57**:90, 1942.

5. Anderson, W. A. D.; Morrison, D. B., and Williams, E. F., Jr.: Arch. Path. **29**:725, 1940; this issue, p. 589.

have undertaken to extend our observations to a species (monkey) in which the pathologic changes of experimental malaria may be compared directly with the effects of injections of ferrihemate.

MATERIALS AND METHODS

Rhesus monkeys were used in all experiments. Twelve monkeys were inoculated with the Rockefeller strain of Plasmodium knowlesi. Frequent examinations by thick smear and parasite counts were made from the time of inoculation until death, the interval ranging from six to sixteen days. Except in 2 cases autopsy was done immediately after death. From the first appearance of parasites in the blood (positive thick smear) to autopsy the time was three to eight days, the usual interval being four or five days.

Five monkeys from the same colony were given intravenous injections of disodium ferrihemate adjusted to p_H 7.6. From one to four injections were made, the amount of ferrihemate per injection varying from 24 to 64 mg. The total amount of ferrihemate injected ranged from 64 to 184 mg. Two monkeys died twenty-three and thirty minutes after receiving the injections. The remaining animals were killed and autopsy performed approximately two weeks after the final injection.

Two normal monkeys were killed and examined as controls.

The methods of preparing the solutions, the technic of injection and the reactions have been described in detail elsewhere.[1]

RESULTS

The pathologic processes in dogs which follow the administration of ferrihemate are formation of thrombi and hemorrhages, deposition of pigment in the reticuloendothelial system and production of renal lesions.[5] In monkeys the lesions following the administration of ferrihemate, while of the same general type, were much less marked than in dogs.

In the monkey which died thirty minutes after an injection of 64 mg. of ferrihemate, the only significant changes at autopsy were small pulmonary and subendocardial hemorrhages, pulmonary edema and phagocytosis of pigment by Kupffer cells of the liver and to a lesser degree in the spleen. In other animals thrombi frequently blocked small vessels. Both pigment and fibrin were usually evident in the thrombi. Vascular occlusions were most common in the lungs, where they were frequently associated with edema and small hemorrhages, but occurred in other locations, also, such as the central nervous system and kidneys. Phagocytosis of injected pigment was always most marked in the liver, and occurred in decreasing amounts in the spleen, the lymph nodes and the bone marrow. In no case was the pigment thus held in the reticuloendothelial system comparable in amount to that in malaria-infected monkeys.

In all the monkeys except those which died very soon after receiving the injections there was histologic evidence of renal damage. In most instances the renal change was slight, consisting of the presence in tubular lumens of an amorphous substance and a pink-staining colloid-like material. Convoluted tubules were mainly involved, but occasionally

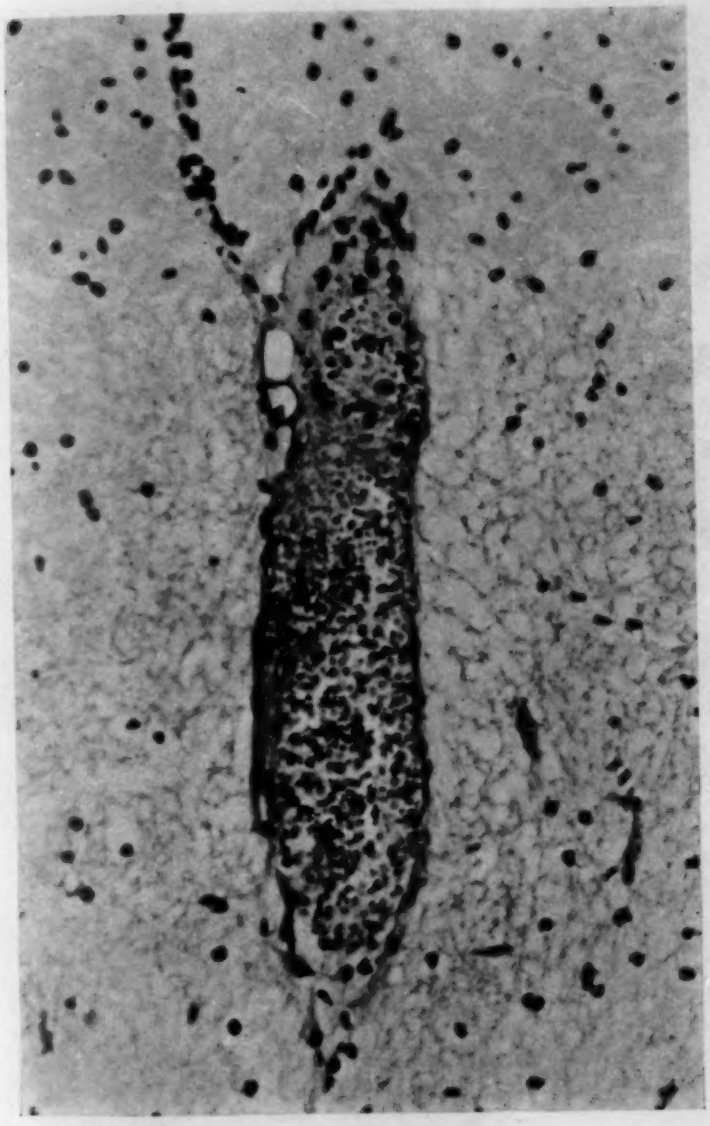

Fig. 1.—Thrombus in a cerebral vessel of malarial monkey 7, composed of fibrin, phagocytic cells, parasites and pigment.

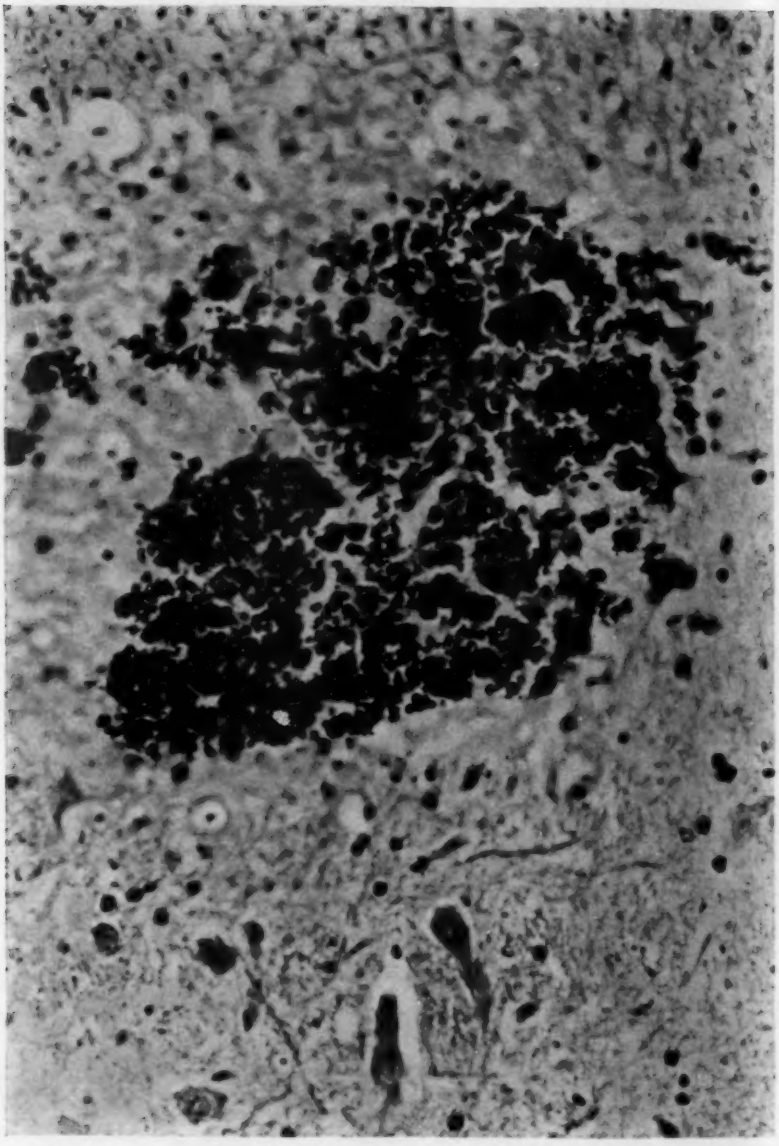

Fig. 2.—Thrombosis and perivascular hemorrhage in the spinal cord of malarial monkey 1, showing accumulation of pigment and phagocytes.

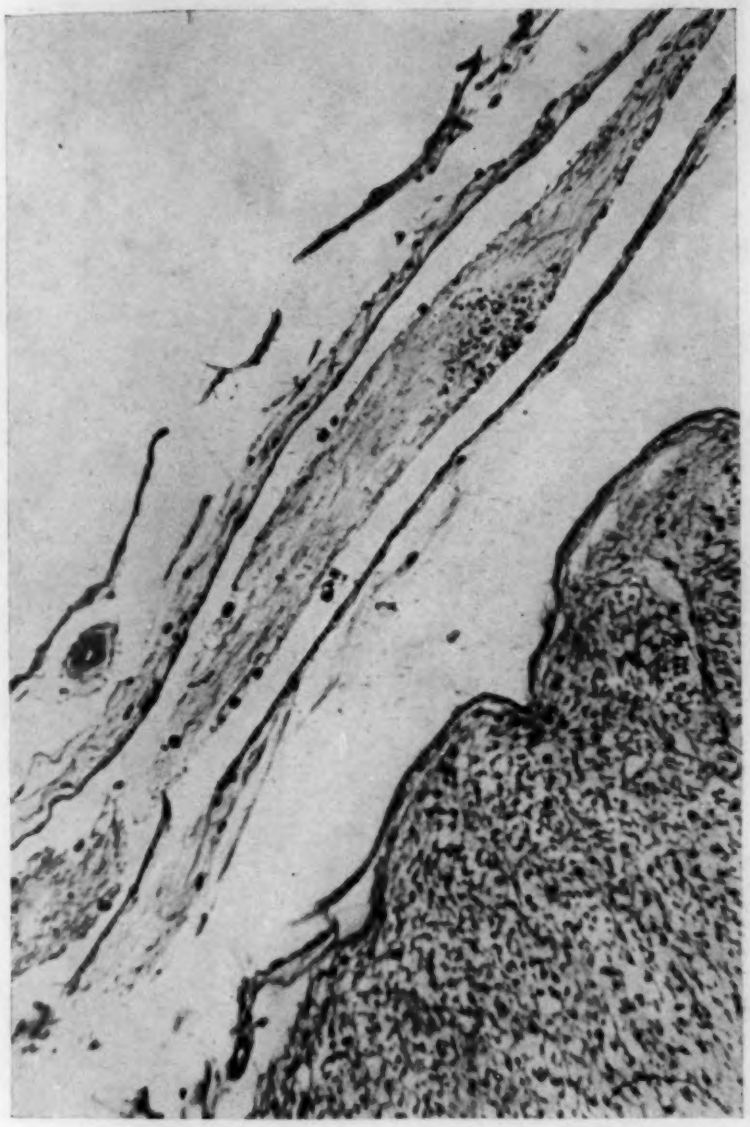

Fig. 3.—Thrombus composed of fibrin with enmeshed parasites in a subarachnoid vessel of the spinal cord of malarial monkey 3.

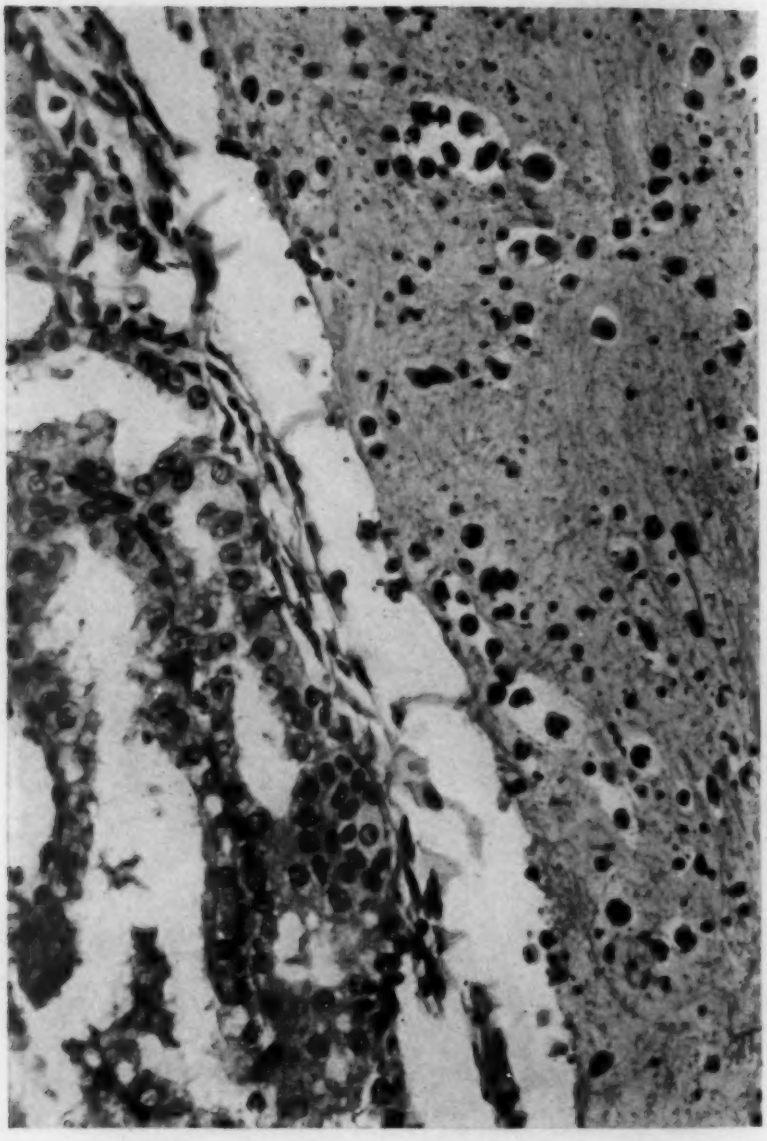

Fig. 4.—Thrombus in a renal vein of malarial monkey 7, showing a meshwork of fibrin around the parasites and cells. The renal tubules show some degenerative change.

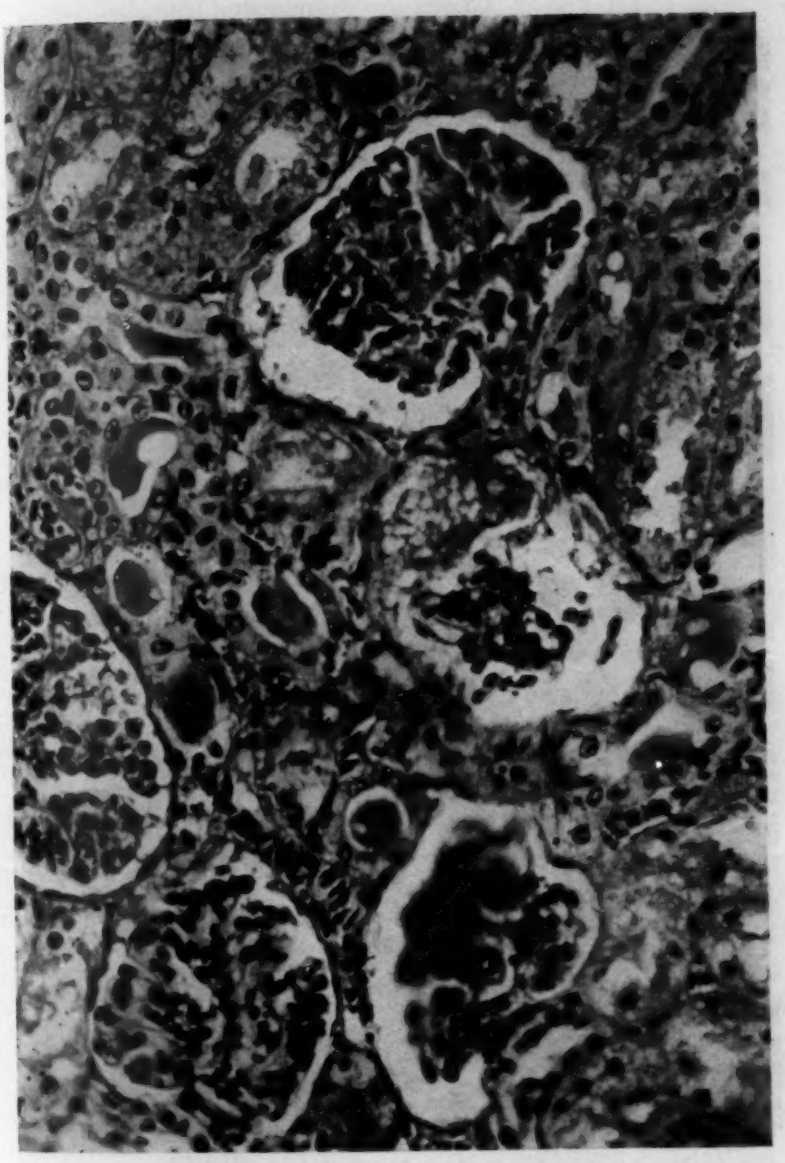

Fig. 5.—Renal cortex of malarial monkey 3, showing tubular degeneration, casts and amorphous material in glomerular spaces and tubular lumens. The capillaries are congested with parasites.

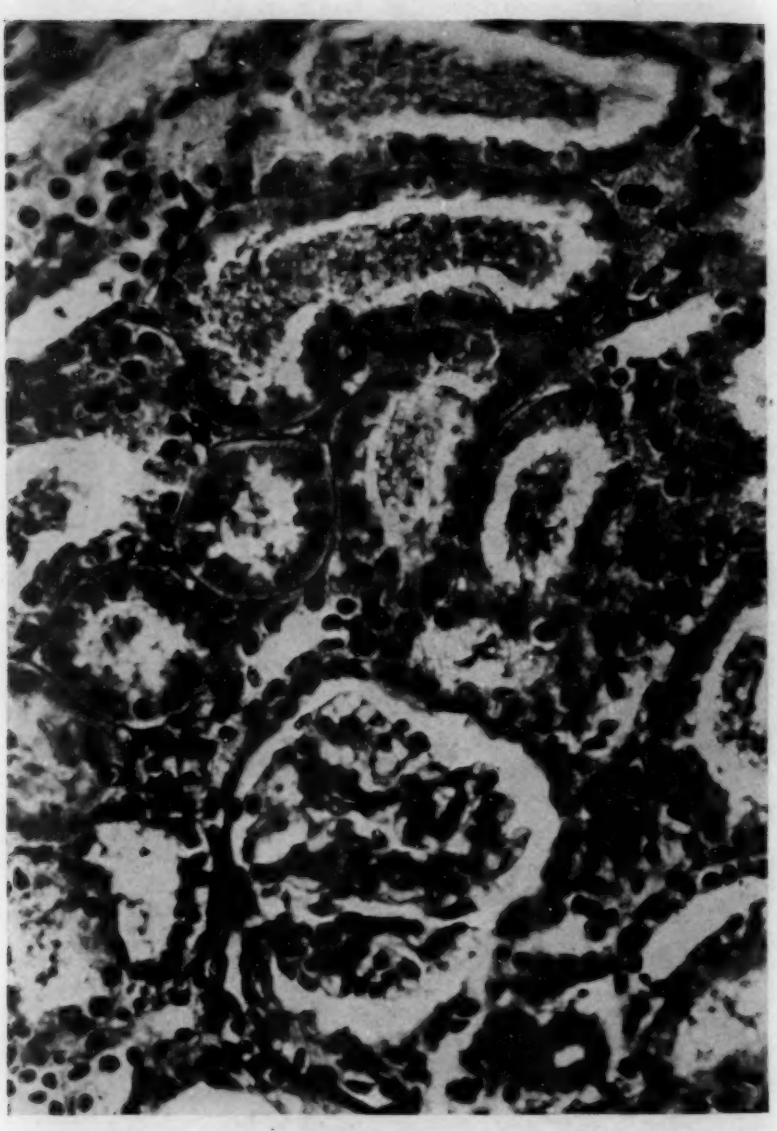

Fig. 6.—Renal cortex of a monkey after the injections of ferrihemate, showing mild degeneration of convoluted tubules and amorphous material in tubular lumens.

Henle's loops or collecting tubules were also affected. Tubular cells showed variable but usually mild degrees of swelling and granularity. Glomeruli exhibited little morphologic change, though usually appearing shrunken and cellular.

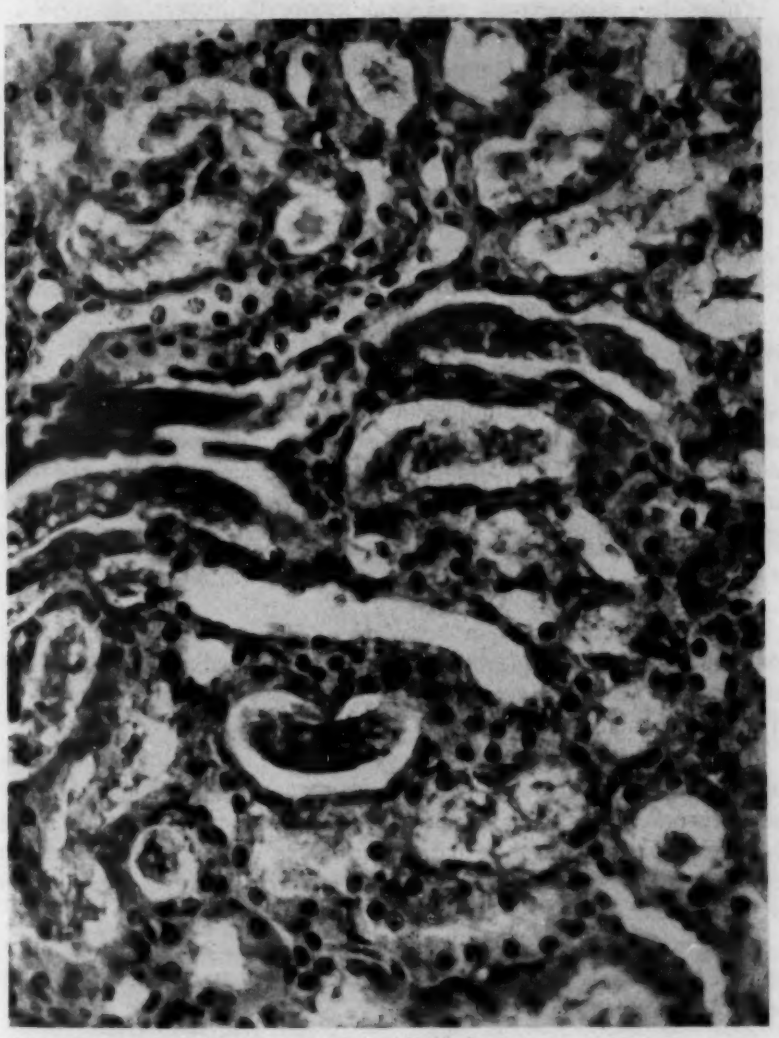

Fig. 7.—Renal tubular degeneration and cast formation following the injection of ferrihemate.

In the monkeys which had been infected with malaria the most striking anatomic changes were related to deposition of pigment. The spleen, most markedly involved, was usually enlarged to several times the normal size and appeared dark grayish black. On the cut surface of the spleen malpighian bodies were prominent as light grayish areas, in

which lack of pigment was confirmed microscopically. The liver, also, was discolored by pigment, which appeared to involve all Kupffer cells. Lymph nodes or bone marrow usually did not exhibit gross pigmentation, but small amounts of pigment were demonstrated microscopically.

Other than phagocytosis and some monocytic hyperplasia there was no marked tissue reaction to the malarial pigment.

Thrombi in small and moderate-sized blood vessels, while not so striking as the dense pigmentation, were almost as constantly found. Such thrombi were observed in the heart, lungs, kidneys, pancreas and central nervous system. Fibrin and pigment in varying proportions composed the thrombi. Both arterial and venous channels were involved.

Renal changes were consistently present in the infected monkeys. Vascular thrombi occurred often, even in larger veins near the pelvis. Degeneration of the epithelium of the convoluted tubules, while always found, varied greatly in severity. In some cases only slight swelling, granularity and irregularity of outline of the tubular lining cells could be demonstrated. In 2 monkeys the tubular degeneration was marked, with prominent hyaline droplet accumulation in the epithelial cells. Amorphous material was frequently evident in glomerular spaces and tubular lumens. Hyaline and granular casts occurred, also, and casts composed of a pinkish red substance. Glomeruli frequently appeared shrunken and cellular, and rarely there were capsular adhesions.

COMMENT

In monkeys, Plasmodium knowlesi infections or injections of ferrihemate cause pathologic changes which are qualitatively similar in many details; changes noted at autopsy in the two experimental conditions differ mainly in degree.

The most significant changes common to the infected animals and the animals given injections of ferrihemate are particularly the widespread thromboses and the renal tubular degeneration and cast formation. These observations suggest that the probable mechanism of injury in simian malaria is anoxemia, due to vascular occlusions, superimposed on the severe anemia already present.

Implication of the parasite pigment as a specific toxic factor is discredited by the fact that pigment is not liberated in soluble form from the parasites.[1]

SUMMARY

In rhesus monkeys infected with Plasmodium knowlesi or given injections of ferrihemate (parasite pigment), the significant changes disclosed at autopsy are multiple thromboses in small vessels, renal degenerative lesions and pigment deposits in reticuloendothelial cells.

Vascular occlusion superimposed on severe anemia, with resultant anoxemia, is the probable mechanism of injury in simian malaria, rather than any direct toxic action of the parasite pigment.

Case Reports

SYNOVIALOMA

PAUL GROSS, M.D., AND DONALD W. CAMERON, M.D., PITTSBURGH

Tumors derived from synovia are uncommon. About 48 such tumors have been reported. In 1936 Knox [1] collected reports of 22 from the literature and added a report of 3 of her own. In the following year Fehr [2] reviewed reports of 24 and added a report of 1. In 1938 Berger [3] listed 24 and reported 5 of his own. However, Berger's review did not include 15 tumors of this type reported by Coley and Pierson [4] in 1937. Since then, reports of 4 others have been published.[5]

The synovial tumors have been termed synovioma, synovialoma, synovial sarcoma and synovial sarcoendothelioma. Most of them have been malignant, and only a few have followed a benign course. (Since the latter have not been traced sufficiently long, some doubt may be expressed regarding the existence of a benign tumor of this type.) It has been a peculiarity of some of these tumors to grow slowly over a period of many years and then to show more rapid growth and metastases. Similarly, pulmonary metastases and death have occurred five to ten years after apparently successful surgical removal of such a tumor.[4] Radiation therapy has been generally of no avail.

In this paper we place on record another of these synovial tumors, one which up to the present has followed an apparently benign course.

REPORT OF A CASE

A white American, 57 years of age, complained of a painful swelling above the left knee. This lump had been present for thirty years. It had been movable and slightly tender but had never caused much distress until one year prior to admission, when it became painful. Several months prior to admission, the swelling became exquisitely tender, and motion of the knee became painful.

There was no significant abnormality except in the region of the left knee. About 5 cm. above the knee, a small rounded, tender, nonmovable and nonfluctuant swelling was found.

At operation, the tumor resembled a mass of varices and was correspondingly vascular. It was dissected from the suprapatellar bursa in its entirety, and the tumor bed was coagulated.

As received in the laboratory, the specimen consisted of a flattened, nonencapsulated piece of pink granular tissue with adherent fat and measured 0.7 by 2 by

From the Institute of Pathology and the Department of Surgery, Western Pennsylvania Hospital.

1. Knox, L. C.: Am. J. Cancer **28**:461, 1936.
2. Fehr, A.: Beitr. z. klin. Chir. **165**:88, 1937.
3. Berger, L.: Am. J. Cancer **34**:501, 1938.
4. Coley, B. L., and Pierson, J. C.: Surgery **1**:113, 1937.
5. Cabot Case 24321, New England J. Med. **219**:204, 1938. von Verebely, T.: Schweiz. med. Wchnschr. **68**:458, 1938. Black, W. C.: Am. J. Cancer **39**:199, 1940. Hutchison, C. W., and Kling, D. H.: ibid. **40**:78, 1940.

4 cm. The tissue was moderately soft and on section exhibited a surface, red, glistening, moist and granular. The impression was gained that the tissue was very cellular. No fibrous tissue stroma could be identified.

Microscopically the tissue, very cellular in some parts and less cellular in others, was honeycombed by slits, oval spaces and blood-containing sinusoids as well as

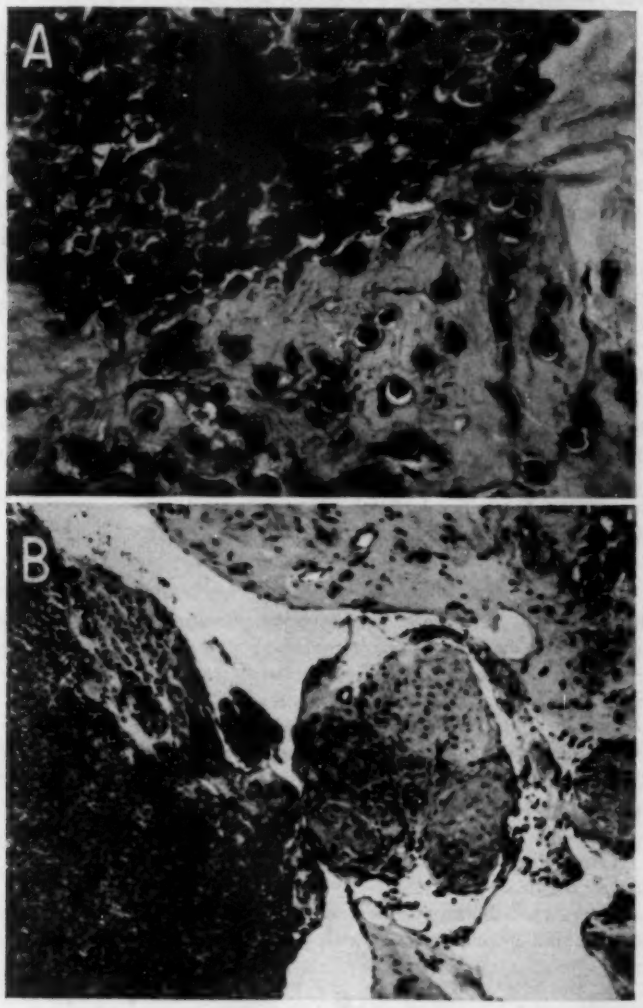

Fig. 1.—*A*, portion of the tumor, adjacent to a densely cellular region which resembles cartilage. The relatively homogeneous matrix and the nuclei lying in lacuna-like spaces are clearly shown. Masson trichrome stain; × 488. *B*, arrangement of cells to form a solid sheet and sinusoidal structures. Masson trichrome stain; × 120.

by small muscular vessels. The cells were of the endothelial type, polygonal or oval and uniform in size; they lay in an amphophilic or slightly basophilic homo-

geneous matrix, which was dyed deep to light green with the Masson[6] stain. Many of the cells possessed clear cytoplasm, although other cells exhibited acidophilic cytoplasmic granulations, which were best demonstrated with the Masson stain. In sections stained by this method even cells with apparently clear cytoplasm showed fine, bright red granulations in a narrow, compact perinuclear zone.

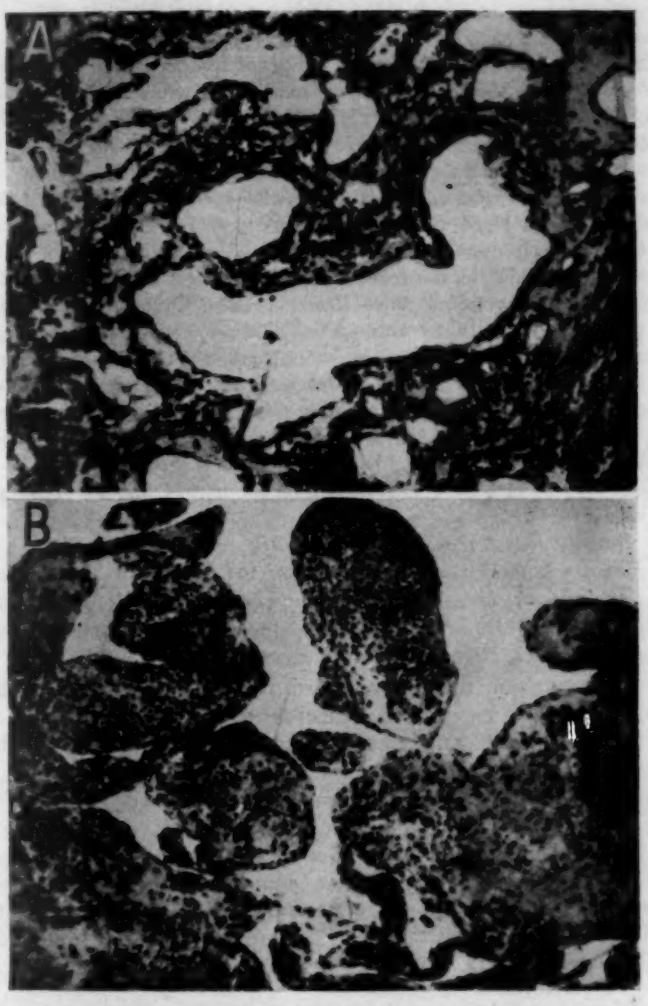

Fig. 2.—*A*, area of the tumor which shows numerous irregular cystic spaces. The lumens are empty. Masson trichrome stain; × 120. *B*, portion of the tumor which shows papillary structures. Some of the tissue resembles cartilage. Masson trichrome stain; × 120.

This zone varied in thickness. It was thickest in cells which had only a narrow peripheral zone of clear cytoplasm.

6. Masson, P.: J. Tech. Methods **12**:75, 1929.

The tumor cell nuclei were oval or rounded and uniform in size and appearance. The nuclear membrane was delicate and sharply defined. The chromatin was finely divided and uniformly distributed. The nucleoli were single and small. No mitotic figures were found.

In some regions cartilaginous tissue was suggested by a homogeneous basophilic matrix in which widely separated cells with clear cytoplasm were found, the latter suggesting lacunar spaces (fig. 1 *A*). In other regions closely crowded cells formed solid sheets (fig. 1 *B*). The bulk of the tissue presented an intermediate appearance relative to the amount of stroma. Except for occasional scattered bundles of loose fine fibrillae, the intercellular substance was homogeneous.

Some of the cells in the less cellular areas were spindle shaped and showed long processes, which were stained red by the Masson method. These cells also participated in the structure of the adventitia of larger vessels.

Many of the oval spaces and most of the slits were empty. Some of the former contained a few red blood cells. The lumens were lined by flat, endothelial-like cells and by the cytoplasmic processes of spindle-shaped cells which surrounded, and were closely applied to, the spaces and slits (fig. 2 *A*). There were occasional small spaces lined by rounded cells. The lumens of these were also empty.

The periphery of the tumor was partially delimited by orderly arranged, relatively acellular dense connective tissue which contained elastic fibers. Beyond this connective tissue, scattered islands of fat were found. Tumor cells disposed in rows and small nests were found on both sides of this connective tissue. Other portions of the tumor periphery were lined by papillae cut in various planes (fig. 2 *B*). These were composed of tumor cells and their matrix. The lining of the papillae was formed by condensations of the matrix and by occasional endothelial-like cells.

No mucin could be demonstrated by the Best mucicarmine method.

The patient was discharged four days after the operation. The incision healed without complications, and the patient returned to work. Follow-up letters, the last more than five years after operation, elicited the information that the patient is well and working. There has been no recurrence.

The structure of the tumor here reported is quite characteristic of synovialoma. The typical features are: endothelial-like cells; a homogeneous, at times cartilage-like matrix; clefts and spaces lined by tumor cells, and papillae.

Although the history, the clinical course and the cellular features indicate a benign tumor, consideration must be given to the failure to demonstrate a capsule grossly and to the fact that microscopically tumor tissue was found beyond the dense connective tissue of the synovial membrane, in the adjacent fat. Attention is also directed to the fact that a number of the reported cases of synovial tumor terminated fatally five and even ten years after apparently successful therapy.

SUMMARY

Synovialoma arising from a suprapatellar bursa is reported. The history of the case indicates slow growth of the tumor over a period of thirty years. The cellular features of the tumor indicate benignancy. The apparent freedom from recurrence five years after surgical excision also supports the impression of benignancy. However, the lack of encapsulation of the tumor tissue and the recognized proclivity of synovial tumors to terminate by pulmonary metastases even many years after apparent cure force a guarded prognosis in this case.

SYPHILITIC ANEURYSM OF THE SUPERIOR MESENTERIC ARTERY

CHAPMAN H. BINFORD, M.D., DETROIT
Passed Assistant Surgeon, United States Public Health Service

Syphilitic aneurysm of the superior mesenteric artery appears to be extremely rare. In 1904 Bacelli [1] described an aneurysm of that vessel in a man aged 34 years who ten years previously had been treated for a penile ulcer which was considered syphilitic. The area involved was explored surgically. The aneurysmal mass, which began at the origin of the vessel, extended along the axis of the latter for a distance of 10 cm. and had a vertical diameter of 7 cm. The author stated that about 20 cases had been reported. He expressed the opinion that the principal cause was syphilis. However, it would be impossible to determine what percentage of these aneurysms were actually of syphilitic origin, because there were no serologic or bacteriologic aids available at that time. Most of the cases referred to were reported long before the histologic recognition of vascular syphilis.

Gifford [2] in 1912 reported an aneurysm of the superior mesenteric artery discovered at autopsy on a Kaffir woman aged 33 years. It measured 2 by 3 by 4 in. and was associated with a fusiform aneurysm of the abdominal aorta. There was no history of syphilis. No mention is made of histologic or serologic studies.

Other references in the available literature covering the last three decades appear to refer exclusively to the so-called mycotic or infectious embolic aneurysm, which was usually associated with vegetative endocarditis.

The aneurysm to be reported was not associated with cardiac disease, but there was definite evidence of syphilis.

REPORT OF A CASE

A Negro man, aged 60, formerly a cook but unemployed for several years, was admitted to the hospital May 28, 1938. The personal history revealed that there had been excessive use of beer and moderate use of tobacco. There had been no previous illnesses of a serious nature. The patient stated that he had not had a venereal disease. The family history was not significant.

The present illness had begun five years before, with shortness of breath and knifelike pain in the chest. This pain sometimes radiated down the left arm. There had been also nausea and vomiting and an occasional fainting spell. These symptoms had gradually increased in severity and in frequency over this period. During the past six months he had been bedridden.

At the time of the initial physical examination it was noted that the respirations were moderately labored and that the patient was more comfortable in a sitting

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From the Laboratory Service, United States Marine Hospital.

1. Bacelli, G.: Policlinico (sez. med.) **11**:301, 1904.
2. Gifford, A. H.: Brit. M. J. **1**:1478, 1912.

position. The heart was thought to be markedly enlarged. A faint systolic murmur was audible. The rate was 98 per minute. The peripheral arteries were firm and tortuous. The blood pressure was 220 diastolic and 130 systolic. Because of the sitting position of the patient, the abdomen was not satisfactorily palpated, but the edge of the liver was observed to be 3 fingerbreadths below the costal margin and tender. The Kahn test of the blood was positive on two examinations. The urine showed a trace of albumin, a few pus cells and specific gravity of 1.013. The leukocytes numbered 14,000. The differential count was normal. An electrocardiographic report indicated mild myocardial damage. The initial diagnoses were general arteriosclerosis and tertiary cardiovascular syphilis.

The subsequent course was as follows:

The patient was treated by rest in bed, sedatives and theophylline with ethylene diamine U. S. P. (aminophylline). Slight symptomatic improvement was observed for a short time. Approximately one month after admission, a pulsating mass about the size of an orange was palpated in the upper middle part of the abdomen. The mass was freely movable and appeared to be connected with the abdominal aorta. A gastrointestinal roentgenologic study, July 5, 1938, demonstrated a persistent crater-like defect in the midpart of the lesser curvature of the stomach. The night following this study right hemiplegia and aphasia developed. However, improvement was rapid, and in one month the paralysis had almost disappeared.

On account of the frequent attacks of severe abdominal pain, accompanied by vomiting, and the roentgenologic evidence of gastric ulcer, a Sippy regimen was tried, but there was no symptomatic improvement.

The pulsating abdominal mass appeared to increase in size. On October 31 the vomitus showed considerable blood, and thereafter the patient frequently vomited bright red or "coffee ground" material. It was noted on November 19 that the liver had become exceedingly hard and nodular. At that time it was the clinical opinion that the nodulation was due to a tumor. As a definite gastric lesion had been observed roentgenologically, a primary neoplasm of the stomach with metastasis to the liver was diagnosed. The pulsating abdominal mass was believed to be a syphilitic aneurysm of the abdominal aorta.

The patient's condition became progressively worse until death, December 19.

Autopsy.—The examination was begun four and three-quarter hours after death. Only the essential observations are reported here.

The lower lobes of the lungs presented numerous pinkish gray nodules, which contained no air. The heart was not enlarged (weight, 275 Gm.). The coronary arteries, while still patent, were tortuous and beaded with many atheromatous plaques. The thoracic and descending portions of the aorta showed several irregularly outlined small plaques of thickened and wrinkled intima and a few areas of atheromatous degeneration.

Lying in the right side of the abdomen, just beneath the pancreas, was a large pear-shaped, moderately firm mass which occupied the position of the superior mesenteric artery. The length was 14 cm. and the average diameter 8 cm. It was largest near the aorta. On longitudinal incision, made through the orifice of the superior mesenteric artery and continued along the lumen until the normal-sized vessel was reached, it was seen that the mass had a thick outer wall more or less continuous with the wall of the artery. Within this outer wall there was a lamellated blood clot, which became more friable toward the center. The lumen

was about 2 cm. in diameter. This aneurysmal mass was adherent to the pancreas and the spleen.

The liver, which weighed 1,500 Gm., presented a coarsely lobular and nodular surface. Many wide scars criss-crossed the organ and between them bulged nodules of pinkish red parenchyma. On section a similar picture of trabecular scarring and

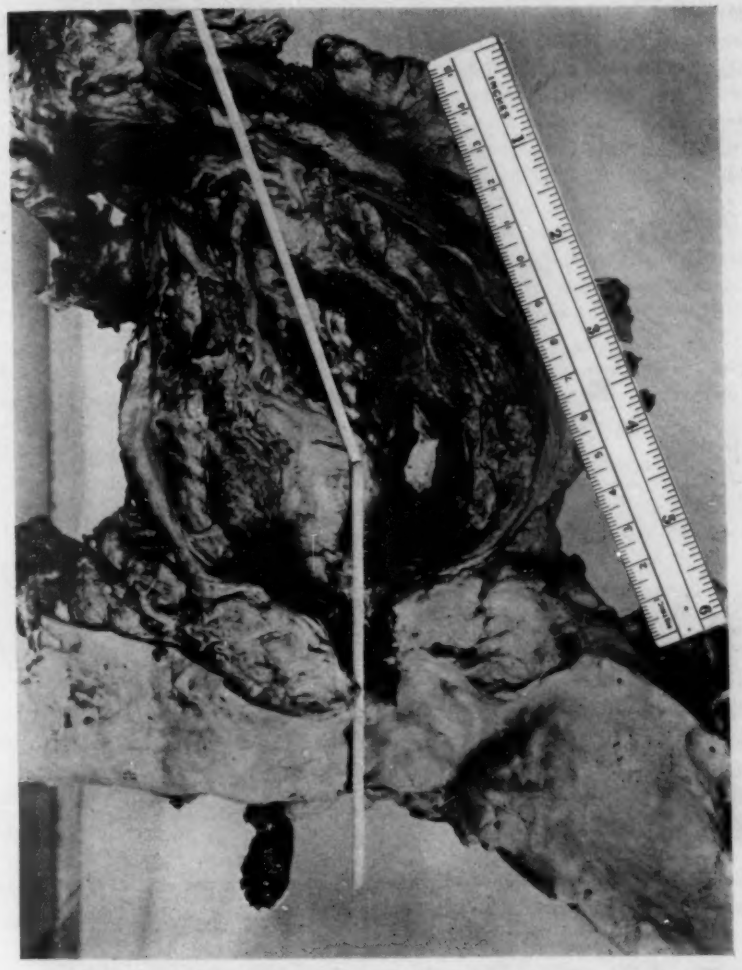

Fig. 1.—Photograph of specimen, including the aorta. The aneurysm has been opened longitudinally. The stick lies in the lumen of the aneurysm and continues in the lumen of the superior mesenteric artery.

coarse nodulation was seen. Occasionally there was a zone about 1 cm. wide in which the parenchyma was softened and of yellow color. The gallbladder was not remarkable.

On the lesser curvature of the stomach about 3 cm. above the pylorus was a gaping crater-like ulcer, which measured 4 cm. across and 2 cm. in depth. The margin was somewhat rolled and dark red. The tissues about the pancreas were thickened and edematous. The lymph nodes of that region measured from 1 to 2 cm. in diameter. The brain was not examined.

Microscopic Examination.—Sections of the ascending aorta presented fairly dense submesothelial fibrosis of the adventitia, accompanied by slight to moderate perivascular lymphocytic infiltration. In the thoracic aorta was a sharply circumscribed calcifying, necrotic and collagenizing lesion occupying the intima and the

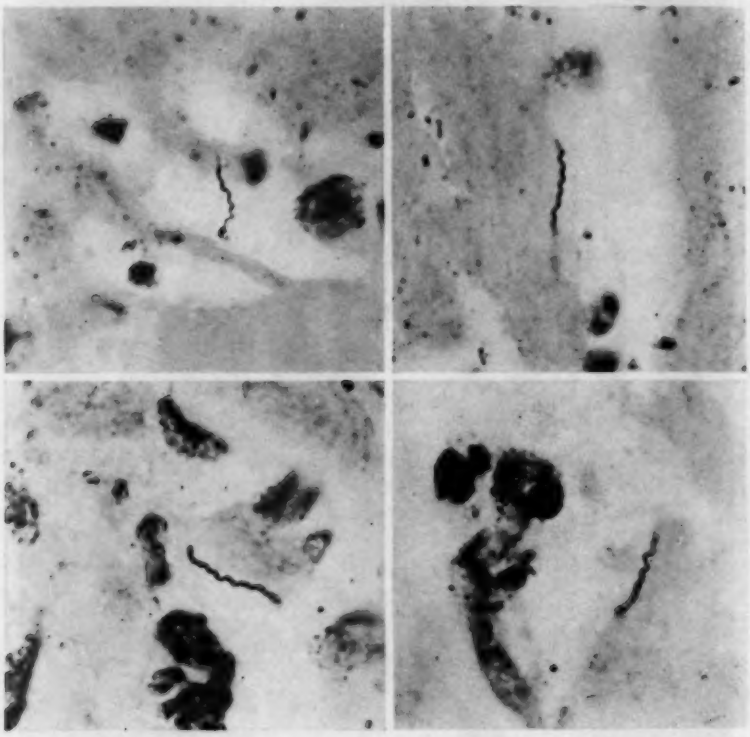

Fig. 2.—Photomicrograph of spirochetes in sections of the wall of the aneurysm. Levaditi's stain; approximately × 1,200.

inner part of the media. The aorta near the orifice of the superior mesenteric artery showed fibrous thickening of the intima and the inner part of the media and, as in other levels, scarring, thickening and some perivascular lymphocytic infiltration of the adventitia. At the orifice of the superior mesenteric artery, in addition to the other changes in the aorta, vascular nodules of lymphocytic and plasma cell infiltrate, measuring up to 1 mm. in diameter, were present in the adventitia and media. In the first part of the superior mesenteric artery was an abrupt replacement of media by whorls of cellular scar tissue.

The proximal part of the aneurysmal sac showed a thin intimal coating of fibrin, scarred remains of media and greatly thickened and scarred adventitia, which was replete with zones of lymphocytic and plasma cell infiltrate. Farther out, the wall of the aneurysm showed an outer zone of lamellated, well vascularized scar tissue infiltrated by many lymphocytes and plasma cells. The inner part of the wall showed organizing blood clot and amorphous material.

In the liver, a widespread coarse trabeculation was effected by copious scar tissue rich in bile capillaries and densely infiltrated by lymphocytes. The capsular scarring was continuous with that of the parenchyma. In many fields the preexisting structural pattern had been almost completely obliterated, but often a centrolobular vein was recognized in the midst of a zone of widened and engorged sinusoids. There were rounded zones of old or recent necrosis ranging up to 1 cm. in diameter. In some necrotic areas and occasionally in the scar tissue, neutrophil infiltration was seen.

Numerous blocks of the wall of the aneurysm were stained by Levaditi's method. In two blocks small areas showed numerous spirochetes. Many were fragmented, and in others the spirals were partially lost, but a fair number presented the morphologic characteristics of Spirochaeta pallida. Sections of liver showed no spirochetes.

The gastric ulcer was of the chronic active type which had eroded almost through the wall. No evidence of tumor was seen. A fair-sized artery in the outer part of the wall adjacent to the ulcer was almost occluded by stenosing intimal proliferation.

Other microscopic observations will be mentioned only in the list of diagnoses.

The final combined macroscopic and microscopic diagnoses were: syphilitic aneurysm of the superior mesenteric artery; syphilitic aortitis in a mild form; syphilitic cirrhosis of the liver (hepar lobatum); gastric ulcer of a chronic active type; aortic arteriosclerosis; moderately severe coronary sclerosis; moderately severe nephrosclerosis of the arteriosclerotic type; chronic active pancreatitis, and chronic active lobular pneumonia.

SUMMARY

A Negro man aged 60 presented at autopsy a large syphilitic aneurysm of the superior mesenteric artery and hepar lobatum. The Kahn reaction of the blood was positive, and Spirochaeta pallida was demonstrated in sections of the aneurysmal wall.

Laboratory Methods and Technical Notes

SIMULTANEOUS SOAP-WAX DEHYDRATION AND INFILTRATION OF THE HUMAN HEART

A Method for Permanent Preservation

R. J. Lebowich, M.D., F. A. Opps and Leonard Procita,
Gloversville, N. Y.

Gross museum specimens are universally preserved by their storage in a glass container filled with a preserving fluid. The wet technic possesses certain definite advantages. It is applicable to all types of material and permits, if necessary, future dissection or preparation of tissue sections. It gives a permanent specimen and preserves the shape and contour of the specimen. However, the use of it is limited by the expense and cost of preparation and mounting, the time and labor involved, the loss of natural colors and the excessive shrinkage. All those who have used the Kaiserling-Pick or Jores methods for preserving museum specimens have been frequently disappointed by the loss of color and the clouding of the fluid through the growth of molds.

The dry technic is carried out by the processes of mummification or infiltration. Mummification consists essentially in the sterilization of an organ or specimen by fixation with an antiseptic fixative followed by complete dehydration. The technic of mummification is now only of historical interest, owing principally to the extreme loss of color and the shrinkage leading to a boardlike consistence and even a distortion of contour and shape, with loss of the finer anatomic landmarks. As a result of these disadvantages it has been abandoned.

The infiltration method is essentially similar in principle to the usual technics employed in the preparation of blocks of tissue for microscopic examination. It consists in the removal of the free and bound water content of the specimen and replacement of this by either paraffin, gelatin or celloidin or some other infiltrating material. Undoubtedly, the most valuable of all infiltrating methods developed to date is that of the paraffin infiltration described by Fredericq.[1] His technic has undergone extensive modifications but no fundamental changes in its basic principles. It has been adapted by Hochstetter[2] to the dry preservation of a wide variety of specimens of human origin and to that of reptiles and other zoologic material.

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1. Fredericq, L.: Bull. Acad. roy. de Belgique **41**:1319, 1876.
2. Hochstetter, F.: Umschau **31**:650, 1927.

Wolhard,[3] Meakins [4] and Gross and Leslie [5] were the first to apply the paraffin infiltration method to the preservation of human hearts. Despite the advantage of permanence, it has not generally replaced the standard Kaiserling-Pick and Jores technics, because of certain inherent limitations. In our own experience, it leads to constant and excessive overdehydration and shrinkage of the right ventricle, so that suitable tissue sections cannot be prepared from the specimen. On the other hand, the left ventricle may be overdehydrated or underdehydrated, depending on the thickness of its wall. Although the topography and the shape of the heart are well preserved, the use of paraffin as the infiltrating medium imparts to the heart a grayish, flat, opaque, washed-out appearance with marked loss of normal color. Kramer [6] recently substituted diglycol stearate for paraffin with favorable results as compared with those from Fredericq's [1] method. However, it is limited by the fact that it varies in chemical composition, and satisfactory tissue sections cannot be prepared from the finished specimen.

The importance and the value of the wet preservation and mounting of the specimens in their "natural colors" is clear: However, this may become a problem because of the expense and the time required for the preparation of the necessary fluid reagents and their use and of the glass museum jars and their maintenance. Therefore, any method which materially reduces the time and the cost of preserving the specimen without the sacrifice of color or the loss of contour and shape is worthy of consideration.

It is the object of this paper to describe a rapid, simple and satisfactory method for the permanent preservation of the human heart.

MATERIALS

A colorless true soap-wax solution composed of monoethanolamine stearate, diethylene glycol and paraffin in molecular proportions [7] acquires a reddish brown color as a result of prolonged oxidation by heat. Immersion of the heart (already fixed in a 4 per cent solution of formaldehyde and partially dehydrated with acetone) in this solution tones the myocardium a reddish brown, the epicardial fat and the aorta a light tan and the veins a blue color.

This method is distinctly superior to the paraffin technic of infiltration in:

1. Preservation of the "color" of the myocardium and valves.
2. Reduction in the shrinkage of the myocardial walls.
3. Rapid simultaneous dehydration and infiltration in vacuo within only two to three days, compared with the ten to fifteen days required by the paraffin technic as applied by Gross and Leslie.[5]

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3. Wolhard, D.: J. Tech. Methods **5**:48, 1915.
4. Meakins, J. C.: J. Tech. Methods **5**:49, 1915.
5. Gross, L., and Leslie, E.: Am. Heart J. **6**:665, 1931.
6. Kramer, F. M.: J. Tech. Methods **18**:42, 1938.
7. Lebowich, R. J.: Arch. Path. **22**:782, 1936; J. Tech. Methods **18**:169, 1938. Moritz, C. E.: Stain Technol. **14**:17, 1939.

4. Economy through the elimination of costly dehydrating, clearing and preserving fluids and glass museum jars.

On the other hand, experience has amply shown that the method is limited by the fact that it is applicable solely to the human heart.

DESCRIPTION OF METHOD

Fixation of the Heart.—Immediately after death, the unopened heart is removed from the pericardial sac; the great basal vessels are severed as high as possible. The heart is now immersed in luke-warm water for thirty minutes to loosen up the blood clots within its cavities. It is then thoroughly washed, for, as a rule, ten minutes in tepid tap water to separate, hemolyze and wash out any postmortem blood clots and fluid blood. Small blood clots still attached to the walls of the cavities are gently removed by means of a forceps without injury to any underlying antemortem thrombi or emboli or to the tissues. The heart should not be opened before formaldehyde fixation is completed, because marked distortion of its normal shape, excessive shrinkage of both the auricular and the ventricular walls and much loss of color will thereby result. The interior of each cardiac cavity is now carefully examined with an electrically lighted nasal speculum, and the presence of any lesions or abnormalities of the valves and the myocardium, as well as their precise color in the fresh, unfixed state, is recorded for future comparison and reference. If desired, it may be of advantage for the same purpose to take a Kodochrome color photograph of the external surfaces. The heart is now weighed.

Formaldehyde fixation may be carried out by either vascular injection or by suspension. With a syringe inserted into the ostiums of the anterior and posterior coronary arteries, small quantities of the 4 per cent solution of formaldehyde are injected at a steady and low rate and pressure to avoid false edema and other artefacts. Both arteries are now individually ligated. Fixation is completed by suspension. For normal hearts, weighing from 250 to 350 Gm., a volume of at least 3,000 to 5,000 cc. of the 4 per cent solution of formaldehyde should be employed.

Should suspension be selected as the method of fixation, the heart is allowed to rest on a bed of absorbent cotton or is suspended in the fixative by strings drawn through the apertures of the pulmonary veins and tied to a glass rod placed over the mouth of a jar. It is not necessary or even desirable to stuff the cavities with cotton in order that their shapes may be retained. After twenty-four hours, the fixative is drained from the heart and replaced by a fresh and equivalent volume of fixative, in which the heart remains for another forty-eight hours. The fixation is completed by a fresh solution, in which the heart remains for another seventy-two hours. The total duration of fixation is six days. Our experience has shown that fixation of the heart by perfusion as recommended by Cross and Leslie is not superior to the technic of simple immersion described here either in point of duration or in efficiency. There is no need for washing out the formaldehyde fixative, as it readily evaporates under negative pressure in vacuo during treatment of the heart. In our experience prolonged fixation of the heart in formaldehyde solution renders it unsuitable for preservation with this method.

As in the case with small tissue blocks, formaldehyde solution effects an appreciable initial swelling with loss of natural colors. However, there is a corresponding disappearance of this initial swelling after treatment of the specimen with acetone and simultaneous dehydration and infiltration in vacuo with soap-wax solution.

At this stage the epicardial fat in the completely fixed heart has an opaque yellowish white color, while the muscle presents a light buff color. The cardiac cavities are now drained completely free of formaldehyde solution and the external surfaces of the heart are dried with a lint-free cloth.

PRELIMINARY DEFATTING AND SIMULTANEOUS DEHYDRATION AND INFILTRATION IN SOAP-WAX SOLUTION IN VACUO

The fat is now extracted from the specimen by suspension for twelve hours in acetone, 5 cc. being employed for each gram of weighed pre-formaldehyde-fixed tissue. After twelve hours, the original acetone solution is replaced by an equivalent volume of fresh acetone. Hearts weighing 400 to 650 Gm. may require an additional twenty-four to forty-eight hours' immersion in acetone to complete the extraction of the tissue lipins, with regular changes at twelve hour intervals. With proper

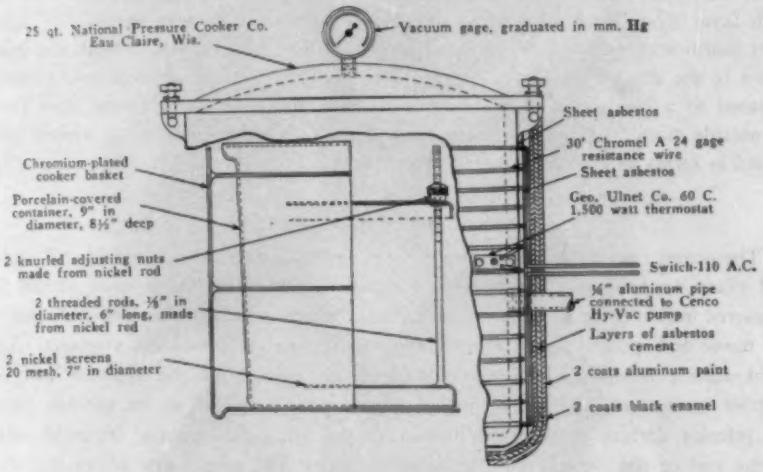

Fig. 1.—Semidiagrammatic sketch of vacuum apparatus utilized for simultaneous soap-wax dehydration and infiltration of human heart in vacuo.

extraction of the fat, the epicardial fat appears pale yellowish white and the myocardium almost white, with a very slight tint of yellow.

The acetone is then drained, and on exposure of the heart to the air for about one hour, the remainder rapidly and completely evaporates. The heart is now transferred to the soap-wax solution for simultaneous dehydration and infiltration in vacuo at 58 to 60 C. for twelve hours. A volume of 3 cc. of soap-wax for each gram of tissue is employed, and after twelve hours it is replaced by an equal volume of fresh solution for another twelve hours.

DESCRIPTION OF APPARATUS

Simultaneous dehydration and infiltration are carried out in a cheap but efficient apparatus (fig. 1) especially devised and constructed for this purpose. It may be used not only for the preparation of human hearts but also for that of small routine blocks of tissue.

A 25 quart air-tight aluminum retort (National Pressure Cooker Company, Eau Claire, Wis.) was remodeled to maintain a constant vacuum in the place of pressure. A vacuum gage, graduated in millimeters, was substituted for the release valve in the cover. The regular safety pressure valve was removed, and its opening fitted with a brass plug. The outside and the bottom of the retort were covered with thin asbestos paper which had previously been soaked in water and then allowed to dry at room temperature. Thirty feet (9 meters) of 24 gage chromel A wire was wound spirally around the outside of the asbestos paper in such a fashion that no two successive spirals came into contact with each other. The ends of the wire were fastened temporarily during the application of the insulating coat of powdered asbestos over the spiral windings. Powdered asbestos mixed with sufficient sodium silicate of a water glass grade to form a semi-solid cement-like paste was now spread on with a spatula in a layer one eighth of an inch (0.31 cm.) thick. Three successive layers of this material were applied, each layer first being allowed to dry before another coat was spread. A 1,500 watt thermostat was now fitted into openings drilled and tapped through the insulation in the side of the retort. One free end of the heating element was securely fastened to a connection of the thermostat, and the other to a second wire from an outside circuit. Connection was made through a single pole throw-switch connected in series with an external 110 volt circuit.

DISSECTION OF HEART

The organ (fig. 2) is now removed from the soap-wax solution while still warm and ready to be opened. For this purpose, a thin, flat, sharp knife should be employed rather than a wedge-shaped knife, which squeezes the soap-wax out of the tissue and spreads apart, as well, the muscle bundles, producing artefacts. The right auricle is opened by cutting between the orifices of the superior and the inferior vena cava, and the auricular appendage is exposed by an incision along the inferior surface of the right border of the heart through the tricuspid valve to the end of the ventricle. The pulmonic valve and pulmonary artery are then opened by an incision beginning at a midpoint of the cut along the right border of the heart, just above the insertion of the anterior papillary muscle, and carried through the left side of the valve. The left auricle is opened by multiple incisions between the orifices of the pulmonary veins, with extension into its appendage.

The left ventricle is opened by a primary incision through the mitral valve, along the left border of the heart between the two papillary muscles and continuing to the apex. Starting at the apex, a second incision is made close to and parallel to the interventricular septum about 1 cm. from the descending branch of the anterior coronary artery. It is continued midway between the left auricular appendage and the pulmonic valve and completed through the aortic valve.

The method of opening the heart described here has proved the most satisfactory in practice. With the organ in the warm state, no more difficulty is encountered in opening it than when it is freshly removed from the body. Difficulties are experienced only when an attempt is made to open the heart after the infiltrated soap-wax has hardened. If desired, only a window exposing an abnormal valve or a portion of injured myocardium may be readily cut out.

While the myocardium and valves are still warm, small coronal and sagittal blocks of tissue of regulation size and thickness for microscopic examination should

be cut. Future additional blocks may be removed from the heart after warming it in soap-wax at a temperature of 58 to 62 C. for about one hour. Tissue blocks of the right ventricle require no further treatment, and after having been embedded in soap-wax solution are ready for sectioning. However, tissue blocks from the left ventricle are but partially dehydrated and infiltrated, and require the same treatment as for regular fixed blocks, viz., one hour in acetone and then immersion in soap-wax solution in vacuo for one hour at 58 to 60 C. at a negative pressure of 250 to 400 mm. of mercury. They are then removed from the dehydrating and infiltrating oven, embedded and sectioned. Satisfactory sections (fig. 3), free from

Fig. 2.—Hypertrophied human heart preserved by soap-wax method, showing incision through right ventricle into pulmonic valve. Large blocks of tissue have been removed from the right ventricle of this specimen for microscopic examination.

excessive shrinkage, are secured which are superior to those obtained with the Fredericq paraffin technic.

If desired, a section of the entire myocardial wall affording a complete microscopic picture of such pathologic processes as myomalacia cordis may be readily prepared. Blocks measuring 5 inches (12.5 cm.) or less in length and 2 inches (5 cm.) or less in width are accommodated in the special Spencer Lens Company knife holder and cut at a thickness of 5 to 10 microns without difficulty, owing to the reduced density of the hardened soap-wax. The usual difficulties which are

encountered in the flattening of the ribbons of large sections on the water bath and which require the use of brushes are eliminated through the use of a bath of 500 cc. or more of 2.5 per cent aqueous chemically pure acacia solution at 48 to 52 C. The mounted sections are of uniform thickness and staining and may be examined for detail with the oil immersion lens.

After cooling, the myocardium appears a light reddish brown color, the epicardial fat and valves, pale yellow and the root of the aorta light tan. The colors are semigloss and are neither dull nor bright. The coronary arteries and their

Fig. 3.—Section of tissue from the wall of the right ventricle shown in figure 2. Note minimum shrinkage of the muscle fibers and the clarity of detail. Hematoxylin and erythrosin stain; × 250.

branches are conspicuously distended with hardened soap-wax and appear light brownish yellow, while the cardiac veins are dark bluish brown. An experience of over two years with this method indicates that the colors remain true and permanent. The semigloss of the colors may be brightened, if desired, by rubbing with a lint-free cloth. Bleaching of the external surfaces of the heart with various bleaching agents is impracticable in that the bleach is temporary.

The specimen should be so mounted that it may be readily available and handled with ease in its demonstration. This is accomplished by inserting a slide clamp

into an area of the uninjured myocardium. This clamp can then be slipped on and off a wooden pedestal with ease and passed around so that each person may examine the heart. When not in use, the specimen on its pedestal is covered with a plastic or other type of transparent jacket to protect it from dust. Light and moisture exert no injurious effects on the colors.

The completed hearts compare favorably in color and shape with control specimens prepared by the Kaiserling-Pick method. However, they suffer from the partial loss of the normal luster of the tissue.

SUMMARY

A rapid and simple technic is described for the dry preservation of the human heart without sacrifice of color through infiltration by a warm liquid soap-wax solution in a specially constructed vacuum apparatus.

It possesses the advantages of economy through the elimination of costly museum jars and preserving fluids and of permanence of preservation.

Tissue sections can be prepared from the finished specimen that are superior to those obtained with the paraffin infiltration technics.

General Reviews

DISSECTING ANEURYSM OF THE AORTA

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CINCINNATI

HISTORICAL DATA

. . . Finally the trunk of the aorta itself, from that place where it sends off its first branches to the upper parts quite to the heart, was both distinguished with spots and marked out into furrows, but these were so confused and irregular that nothing but a perpetual and very great irregularity of that surface appeared. Yet, besides this, a kind of ulceration, as it were, was found about two inches above the semilunar valves, where the artery looks toward the right and posterior parts; and in that ulceration were three or four very deep foramina, very near each other, each of them of the bigness of a lentil, but of an angular form rather than round. From these foramina, winding sinuses were carried obliquely outwards and reached to the external lamina of the aorta; which was in that place, therefore, of a brownish color mixed with red, as if in consequence of inflammation, and became much thickened by a great flow of moisture; and in the middle of that redness, the lamina being at length lacerated, the blood had made a way for itself into the pericardium, by a foramen similar to the internal foramen, and almost the same magnitude. . . .

Thus wrote Morgagni [1] of the changes observed at autopsy in a woman of more than 30 years of age who died suddenly in the year 1708. Several equally clearcut descriptions of dissecting aneurysms are recorded by the same author,[1b] but with respect to cases reported prior to this date exact identification of the type of aneurysm portrayed is difficult. Scarpa [2] stated that it was not until about the year 1557 that any certain knowledge was obtained as to the occurrence of "internal aneurysms"; at that time Vesalius described a pulsating tumor which developed on a man's back near the spine after a fall from a horse. Scarpa discredited an early opinion, held by Fernelius, that aneurysms

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1. Morgagni, G. B.: (*a*) De sedibus et causis morborum per anatomen indagatis libri quinque, Venice, ex typog. Remondiniana, 1761, ep. 26, art. 21; The Seats and Causes of Diseases Investigated by Anatomy, translated by B. Alexander, London, A. Millar & T. Cadele, 1769, vol. 1, p. 808; (*b*) ep. 26, art. 15 and 17, and ep. 27, art. 28.

2. Scarpa, A.: A Treatise on the Anatomy, Pathology, and Surgical Treatment of Aneurysm, translated by J. H. Wishart, Edinburgh, Mundell Doig & Stevenson, 1808.

were due to a morbid distention of all the coats of an artery and advocated the concept advanced by Sennertus, in 1628, that internal aneurysms resulted from rupture of the internal coats of an artery with elevation and distention of the external coat by extravasated arterial blood, stating that these beliefs were based on solid anatomic knowledge and mature reflection.

A century later Nicholls [3] argued that any impediment to the progressive motion of blood within a vessel greatly raising the intravascular pressure might occasion either a distention without rupture or a rupture of the internal coat. He described experimental overdistention of the pulmonary artery at the autopsy table, resulting in bursting of the internal coats with formation of an aneurysmal outpouching of the external coat. The internal coats, he believed, were less resistant and apt to give way because of the anatomic disposition of their fibers. In 1761 Nicholls [4] reported his necropsy observations on the body of King George II, who a year before had succumbed to a rupture of the right ventricle while at stool. He demonstrated a dissecting aneurysm within the coats of a dilated aortic arch and a large transverse rent in the intimal coat and offered these findings as substantiating evidence for his aforementioned hypothesis on the effects of greatly increased intravascular pressure. He further ascribed the rupture of the right ventricle to compression of the pulmonary artery by the contiguously dilated and dissected aorta.

Maunoir,[5] in 1802, clearly described dissecting lesions not only of the aorta but also of the pulmonary artery. General recognition of dissecting aneurysms appeared lacking, however, until Laennec's [6] classic work, "Traité de l'auscultation médiate," appeared in 1819. Laennec spoke of "anévrysme disséquant" and appears to have been the first to employ the term. Three years later Shekelton [7] described cases of "healed" dissecting aneurysm in which circulation was reestablished through a second opening of the intramural lesion into the aortic lumen.

The careful investigations of Peacock [8] on the nature of the disease as a distinct entity appeared in 1843 when he reported on 19 cases and discussed the subject fully. Two decades later he was able to collect 80 cases from the literature.[9] Since that time reviews treating various

3. Nicholls, F.: Phil. Tr. Roy. Soc. London **35**:440, 1728.

4. Nicholls, F.: Phil. Tr. Roy. Soc. London **52**:265, 1763.

5. Maunoir, J. P.: Mémoires physiologiques et pratiques sur l'anévrisme et la ligature des artères, Geneva, J. J. Paschoud, 1802.

6. Laennec, R. T. H.: Traité de l'auscultation médiate, Paris, J. A. Brosson & J. S. Chaudé, 1819, vol. 2, p. 411.

7. Shekelton, J.: Dublin Hosp. Rep. **3**:231, 1822.

8. Peacock, T. B.: Edinburgh M. & S. J. **60**:276, 1843.

9. Peacock, T. B.: Tr. Path. Soc. London **14**:87, 1863.

aspects of large numbers of collected cases have been published by Bostroem,[10] Flockemann,[11] Schede,[12] Frei,[13] Crowell,[14] Schnurbein,[15] Pannhorst,[16] Shennan [17] and McGeachy and Paullin.[18] At the present time the total number of cases recorded in the literature is around 500. The first clinical recognition of the disease during life was reported by Swaine and Latham,[19] in 1856. A recent article [20] places the total number of published cases in which the condition was correctly diagnosed before death at 33. This figure undoubtedly represents an underestimation of the number of cases correctly diagnosed at the bedside, as many of them are never reported in the literature.

INCIDENCE

Present statistics on the incidence of dissecting aneurysm vary somewhat. In reports of the larger autopsy series, which serve as reliable indicators, the figures range between 1 in 200 and 1 in 552 cases. An average of these figures places the incidence at 1 in 381 cases,[21] which seems to be a fair estimate. A review of the files of the Cincinnati General Hospital for approximately twenty years revealed 24 dissecting aneurysms among 11,131 autopsies, an incidence of 1 in 464 cases.

It is reported that males are affected twice as often as females, but Shennan pointed out that the incidence in the different decades of life shows a less striking variation. Thus in the third and seventh decades the percentage of males was found to be greater than that of females while in the eighth and ninth decades the percentage of females was higher. The highest age incidence occurs between the fourth and seventh decades, although hardly any age is exempt. A dissecting aneurysm of the descending thoracic aorta with fatal rupture in a 14 month old boy and another in a 10 year old boy were reported by Frei. Wolff [22] described a remarkable case of diffuse destruction of elastic and collagen fibers with mucoid accumulations and focal hemorrhages in the

10. Bostroem, E.: Deutsches Arch. f. klin. Med. **42**:1, 1887.
11. Flockemann: München. med. Wchnschr. **45**:847, 1898.
12. Schede, F.: Virchows Arch. f. path. Anat. **192**:52, 1908.
13. Frei, C.: Ueber das Aneurysma dissecans aortæ, Inaug. Dissert., Zurich, J. J. Meier, 1921.
14. Crowell, P. D.: J. A. M. A. **77**:2114, 1921.
15. Schnurbein, F.: Frankfurt. Ztschr. f. Path. **34**:532, 1926.
16. Pannhorst, R.: Deutsches Arch. f. klin. Med. **175**:115, 1933.
17. Shennan, T.: Dissecting Aneurysms, Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.
18. McGeachy, T. E., and Paullin, J. E.: J. A. M. A. **108**:1690, 1937.
19. Swaine and Latham: Tr. Path. Soc. London **7**:106, 1856.
20. Rogers, H.: Am. Heart J. **18**:67, 1940.
21. Holland, L. F., and Bayley, R. H.: Am. Heart J. **20**:223, 1940.
22. Wolff, K.: Virchows Arch. f. path. Anat. **285**:369, 1932.

aortic media of a 12 day old infant. True dissection of the coats with formation of an intramural hematoma did not occur, though the changes described suggest the early development of such a process. Rokitansky's case [23] concerned a cyanotic boy of 8 years, who at autopsy was observed to have a congenital narrowing of the aorta with an associated ventricular septal defect and a distortion of the pulmonary artery. Wasastjerna [24] reported on a case of dissecting aneurysm of the aorta of a 13 year old boy with associated coarctation of the aorta. Klotz and Simpson,[25] in classifying 42 cases in which the patients were below the age of 40 years, found 7 cases in which the patients were between 11 and 20 years of age. At the other extreme of life was the patient in the case cited by Shennan,[17] a woman nearly 100 years of age.

PATHOGENESIS

Despite the large number of cases reported and the different approaches adopted by various investigators studying this disease, some lack of unanimity is evident regarding both the genesis of the process and the nature and the evolution of the lesion within the coats of the aorta. Before proceeding further, a few words on the term "dissecting aneurysm" seem apropos. This term is ordinarily restricted to a condition in which the vascular coats, usually the outer and the middle, are separated from one another by an accumulation of blood. This frequently communicates with the lumen through a tear of the inner coats and ruptures through the adventitia into the pericardium, the pleura, the peritoneal cavity or other places. The term "spontaneous rupture," on the other hand, commonly implies a complete transverse, less often an incomplete or longitudinal, parting of the vessel wall rather than separation of the individual coats. Such an event occurs rarely without some degree of dissection and intramural hemorrhage, and conditions which predispose to dissection are the same as those which permit rupture to occur. Hence the terms "dissecting aneurysm" and "spontaneous rupture" of the aorta are frequently employed interchangeably and have been accepted by usage. The method by which such a process is initiated has been the subject of considerable controversy. Excluding such conditions as violent traumatic injury, acute mycosis of the vessel wall, erosion of the vessel by a neighboring foreign body and extension of an inflammatory or a neoplastic lesion from a structure adjacent to the aorta, two hypotheses have come into consideration concerning the events leading to dissection of the vessel coats.

One of these maintains that a sudden or a sustained marked increase of intravascular pressure is in itself sufficient to cause rupture and dis-

23. von Rokitansky, C.: Denkschr. d. k. Acad. d. Wissensch. **4**:52, 1852.
24. Wasastjerna, E.: Ztschr. f. klin. Med. **49**:405, 1903.
25. Klotz, O., and Simpson, W.: Am. J. M. Sc. **184**:455, 1932.

section of a healthy aorta. The other postulates primary damage to one or more of the aortic coats as prerequisite to dissection, regardless of pressure changes.

In attempting to ascertain the amount of pressure normal arteries are capable of withstanding, Klotz and Simpson [25] subjected aortas from persons between the ages of 20 and 40 years to pressures of 1,000 mm. of mercury without succeeding in rupturing the coats. Oppenheim,[26] however, caused an apparently healthy aorta from a 39 year old woman to rupture at a pressure of about 2,070 mm. of mercury. Another aorta from a 62 year old woman ruptured at the site of a calcified plaque at 790 mm. of mercury. In order to exclude the possibility that the fresh aorta unaffected by postmortem rigidity might burst under less pressure than one in which rigor had set in, Schnurbein [15] applied this test to the aorta of the rabbit. He found that the aorta from a freshly killed animal and that from one dead for some time would rupture at about the same pressure. Employing living animals to determine whether rupture of the aorta would occur more easily if the intravascular pressure was raised suddenly or was long continued, he gained the impression that the vessel would stand about one-tenth more pressure if this was exerted suddenly than it would if the pressure was long continued. A description of his method was omitted from his report. Moritz [27] was unsuccessful in his attempts to produce rupture of the aorta in living young rabbits by injecting a fluid solution into the vessel under high pressure. When pressures of 800 to 1,200 mm. of mercury were attained, a sudden drop in pressure occurred, which was due to rupture of the portal vein or one of its tributaries following transmission of pressure which had been too rapid to produce an effect on the aortic wall. It appears unlikely from the evidence at hand that increases of pressure alone could reach sufficient magnitude in human subjects to cause actual dissection of the coats. The long-standing hypertension frequently observed associated with dissecting aneurysm never attains more than a fraction of the pressure necessary to tear the aorta experimentally. Furthermore, those cases of long-standing hypertension are most common in the age past 40 years, when some degree of muscle and elastic tissue degeneration of the aorta is practically universal, hence the abnormally elevated pressure could hardly be considered the sole factor in producing dissection. It may be argued, on the other hand, that increased vascular pressure associated with coarctation of the aorta is common in the earlier decades of life and that in patients with this condition rupture of the vessel occurs rather frequently. Abbott and Hamilton [28] in analyzing

26. Oppenheim, F.: München. med. Wchnschr. **65**:1234, 1918.
27. Moritz, A. R.: Am. J. Path. **8**:717, 1932.
28. Abbott, M. E., and Hamilton, W. F.: Am. Heart J. **3**:381, 1928.

a series of 200 cases of coarctation of the aorta noted that spontaneous rupture occurred in 38. In 33 of these the rupture occurred proximal to the site of coarctation and in 5 below it. In the former group an intimal tear was consistently found a short distance above the valve cusps, and in the great majority of cases this communicated with a dissecting aneurysm extending between the medial fibers or between this coat and the adventitia, perforating secondarily into the pericardium or an adjacent viscus. In 29 of the 33 cases the ascending aorta was dilated, sometimes widely. Among 13 cases in which the vessel was examined microscopically there were 12 in which pronounced medial changes were found in areas adjacent to but not involved in the rupture. In the remaining case the presence of histologic abnormalities in the path of the dissection itself cannot, of course, be excluded on the basis of absence of medial damage elsewhere. Such almost constant changes in the wall of the vessel suggest strongly that the role played by increased intravascular pressure in these cases is of secondary importance. Reports of dissecting aneurysms unassociated with vascular anomalies in which hypertension was alleged to be the sole cause for dissection also seem open to the suggestion that further microscopic scrutiny might have revealed such anomalies.[29] It is noteworthy that the early and widely quoted concept of Thoma,[30] postulating a physiologic weakness of the arteries, or angiomalacia, in which molecular disintegration of the coats, though not discernible histologically, predisposed to rupture, was modified in a later publication. Here he admitted that better investigative methods had made it possible to demonstrate microscopically various degenerative changes taking place even in the early stages of angiomalacia.[31]

Returning now to the second hypothesis which holds that damage existing in the aortic wall is of primary importance in the formation of a dissecting aneurysm, considerable supporting evidence has accumulated, although opinions differ as to which arterial coats are chiefly concerned, as well as to the physiologic importance of each and the significance of their individual tissue components.

The question of the relative functional importance of each of the aortic coats can be approached best by visualizing the histologic structure. Orsós'[32] detailed study on the normal media emphasized a close interrelationship of the various elements of this broad coat. He demonstrated the elastic ground lamellas embedded intimately in collagenous tissue, surrounded by layers of precollagenous fibrils which can be

29. Gager, L. T.: Ann. Heart J. **3**:489, 1928. Arenberg, H.: ibid. **8**:217, 1932.
30. Thoma, R.: Virchows Arch. f. path. Anat. **116**:1, 1889.
31. Thoma, R.: Beitr. z. path. Anat. u. z. allg. Path. **66**:377, 1920.
32. Orsós, F.: Verhandl. d. deutsch. path. Gesellsch. **26**:365, 1931.

impregnated by silver stains. In the interlaminar spaces between two layers of precollagenous fibrils are the muscle cells, enveloped by a delicate perimysium and fine elastic fibrils. The spaces in this fibrillar-muscular layer are filled from birth with mucoid material which, he held, acts as an embedding lubricant for the other elements and facilitates their action.

The intima, on the other hand, is less complex in structure and while possessing a prominent internal elastic lamina is made up of rather delicate branching elastic fibers, small numbers of muscle cells and a few collagenous fibers; not infrequently it exhibits marked regressive changes early in life, and in advanced age these may attain a degree suggesting total loss of functional capacity, despite which circulatory compensation is adequately maintained. The adventitia marking the opposite medial boundary is the source of the important vasa vasorum but contains only loosely arranged collagenous and elastic fibers and sparsely scattered smooth muscle cells. Hence it becomes evident that the compact media alone is structurally endowed to supply the tensile strength needed for normal or excessive circulatory strain. Because of its physiologic importance it is also the site likely to respond to relatively minor anatomic injuries with a maximum disturbance of work efficiency.

HISTOLOGY OF THE AORTIC LESIONS

The early investigative work of Wiesel [33] on medial damage to arteries during acute infectious diseases was carried out without reference to the question of the formation of a dissecting aneurysm, yet it established a firm basis for further study in this direction. This author noted primary degenerative changes limited to the medial coats of both the large elastic and the smaller peripheral arteries of nonsyphilitic young persons dying a short time after the onset of fulminating acute infections. The earliest vascular change encountered was the presence of a homogeneous interstitial substance between the elastic fibers and the musculature of the media. Extensions of this process resulted in encroachment on and destruction of either one or the other of these elements. In one group of cases including cases of diphtheria, typhoid fever, influenza and pneumonia, the elastic fibers appeared to be chiefly involved, while in another group including cases of scarlet fever and septic and pyemic states the medial muscle elements were mainly destroyed. In both groups there was a noteworthy lack of any cellular inflammatory exudate. Calcification was not found in the necrotic areas, and the changes were followed in some instances by scar tissue replacement and in others by attempted regeneration of elastic and muscle

33. Wiesel, J.: Ztschr. f. Heilk. **27**:262, 1906; **28**:69, 1907.

tissue. Wiesel expressed the belief that these nonexudative medial changes might lead to secondary intimal atherosclerosis and offered this as an explanation of childhood atherosclerosis following infections. Stoerk and Epstein [34] examined medium-sized and large arteries taken from young persons who had died of influenza in the epidemic of 1918-1919. They found the elastica interna to be the portion of the vessel most vulnerable to attack. The muscle cells were next involved in a reactionless necrosis, which was not followed by scar formation but instead by production of young muscle elements, which in places filled the gaps left by the ruptured elastica interna of the intima.

Freedman [35] described a chance finding of an isolated, rather prominent cystlike area in the medial coat of a carotid artery of a 60 year old patient who died of acute lobar pneumonia. The cystlike area was filled with gelatinous semisolid material and was unassociated with any cellular reaction. Microscopically, the changes appeared to begin in the neighborhood of the elastic laminae and, with increase in size of the cyst, involved the muscle fibers. Here there is little doubt that the isolated lesion of the vessel antedated and was unassociated with the pneumonic process. Long before the presence of such medial changes was linked with the development of a dissection of the vessel coats, Rokitansky [23] offered a classification of dissecting aneurysms based on changes of several types in the individual coats of the aorta. In one group of cases the adventitial coat was described as thickened, unusually vascular and in a state of chronic inflammation, permitting easy separation from the essentially intact inner coats, which on splitting became dissected from the adventitia by the inflowing blood. In another group the adventitia was described as normal while the medial coat was unusually brittle, degenerated and studded with heaps of fat molecules. Here a rent beginning in the inner media was held responsible for separation of the outer media and adventitia following an influx of blood through an intimal tear. While histologic data on the latter group of cases is inadequate, the description suggests a primary type of degenerative noniflammatory medial change producing dissection of the aortic wall.

Detailed investigation of the spontaneous medial lesions leading to dissolution and separation of the aortic coats reveals changes of several different kinds, some of them apparently representing different stages of the same process and others appearing quite distinctive in their distribution and evolution. Common to the great majority of these changes is a process which is essentially degenerative in nature, the mode of appearance of which cannot be explained by disease or obstruc-

34. Stoerk, O., and Epstein, E.: Frankfurt. Ztschr. f. Path. **23**:163, 1920.
35. Freedman, A.: Montreal M. J. **38**:583, 1909.

tion of the vasa vasorum or by mechanical interference from a diseased intima. Thus Moriani,[36] investigating a dissecting aneurysm of the descending thoracic aorta of a 50 year old woman, found pronounced intimal changes limited to a small portion of the abdominal aorta while a transverse intimal tear was present in the ascending thoracic aorta. The medial coat was the site of numerous small scattered foci of fatty degeneration involving the elastic lamellas, the adjacent muscle cells and the supporting precollagenous lattice fibrils. Coalition of areas of fatty degeneration sometimes resulted in the formation of clefts filled with amorphous debris. Such medial clefting was most pronounced in the region of the large tear in the aortic arch, but similar changes were present in far removed zones. One such isolated area of splitting was partly filled with blood from a ruptured vas vasi, while the overlying intima was unbroken. Another medial change considered of great importance in preparing the path for dissection was hyaline degeneration of the interlamellar connective tissue. Moriani also stressed the physiologic importance of damage to the precollagenous lattice or cementing fibrils, looking on their destruction as a blow to the inhibitory or checking force exerted against overdistention of the elastic fibrils. Benda [37] had previously noted a constant breaking-up of the elastic fibers of the media in dissecting aneurysm but found them replaced by richly vascular connective tissue.

An article by Babes and Mironescu,[38] which appeared almost simultaneously with Moriani's report, contributed further information on the nature of the medial changes leading to dissecting aneurysm. In the case they reported the changes consisted chiefly of scattered spindle-shaped clefts within the media, surrounded by thickened elastic fibers, fusing in some areas to form rather large spaces. These contained colorless serous or glossy material lined by elastic fibrils. Fatty changes were found localized here and there about these elastic fibrils, and some scattered groups of muscle cells were impregnated with calcium deposits. Portions of the damaged media showed striking proliferation of fibroblasts and accumulation of lymphocytic and enlarged endothelial cells. In other areas occasional small nodules of granulation tissue surrounded lymph vessels. Foci of granulation tissue were also scattered in the adventitia, and some hemorrhage was noted about the vasa vasorum. While the inflammatory elements here contrast sharply with the purely degenerative changes in Moriani's case, the predominant lesion appears to be retrograde in nature, complicated by some patchy cellular reaction.

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36. Moriani, G.: Virchows Arch. f. path. Anat. **202**:283, 1910.

37. Benda, C.: Ergebn. d. allg. Path. u. path. Anat. **8**:196, 1902.

38. Babes, V., and Mironescu, T.: Beitr. z. path. Anat. u. z. allg. Path. **48**:221, 1910.

Similar but less pronounced cellular activity was present in Krukenberg's [39] methodically studied case, and it was associated with marked degenerative medial lesions.

Another variation in tissue damage was brought out by Gsell's [40] careful observation in 8 cases of dissecting aneurysm. The lesion was characterized by focal areas of necrosis involving principally the muscle cells and progressing from simple loss of nuclei to complete destruction of the cells and scattering of nuclear fragments about the zones of necrosis. A loss of connective tissue nuclei paralleled this change or appeared slightly later, and the elastic and collagen fibers showed subsequent flattening, crowding together and degenerative changes. Regenerating networks of delicate elastic fibers were found in the necrotic foci, and occasionally some newly formed smooth muscle cells were seen; within the larger regenerative foci were thin-walled capillaries. Some foci of tissue destruction contained clefts filled with mucoid ground substance with embedded spindle-shaped nuclei. Thinning of the media, often to a marked degree, as a result of the tissue damage was found to be compensated for by a corresponding increase of connective tissue in the adventitia. The adventitial vessels, however, were patent and free from plugging except in 1 case, where they were involved in an extension of a contiguous tuberculous inflammatory process. The intimal coat was constantly narrow and even depressed over the areas of medial damage. Cellular reactions were strikingly absent throughout. Gsell emphasized the weakening effect exerted by the muscle necrosis on the resistance of the aortic wall. This supported Benninghoff's [41] contention that the muscle fibers of the aortic media insert by delicate end processes into the elastic lamellas and thereby act in the capacity of a regulator of the elastic structure, maintaining the caliber of the lumen during variations in blood pressure. Loss of this adaptive function through muscle cell damage would permit overstretching of the elastic fibrils and widening of the lumen, thus preparing the way for subsequent tearing.

Erdheim,[42] investigating a small dissecting aneurysm of the supravalvular portion of the aorta of a 76 year old man, commented on a small number of acellular areas of focal necrosis in the outer medial coat. These he regarded as probable phenomena of aging and of minor significance. He upheld as of prime importance in leading to dissection of the wall numerous scattered areas of mucoid degeneration in the medial coat of the thoracic aorta. In a previous report [43] he had observed

39. Krukenberg, E.: Beitr. z. path. Anat. u. z. allg. Path. **67**:329, 1920.
40. Gsell, O.: Virchows Arch. f. path. Anat. **270**:1, 1928.
41. Benninghoff, A.: Ztschr. f. Zellforsch. u. mikr. Anat. **6**:348, 1927.
42. Erdheim, J.: Virchows Arch. f. path. Anat. **276**:187, 1930.
43. Erdheim, J.: Virchows Arch. f. path. Anat. **273**:454, 1929.

two distinct lesions in the media: sparsely scattered foci of necrosis characterized principally by nuclear destruction; more numerous distinctive focal areas of tissue loss in which elastic and collagen fibers were more or less simultaneously effaced, the muscle cells occasionally persisting in fields of cleft and cyst formation. He did not regard the latter lesion as a proved sequel of the less commonly observed necrotic foci as suggested by Gsell's earlier observations, but felt that their relationship was undecided from the evidence at hand. In his second article he elaborated on the importance of the mucoid changes and postulated that these were forerunners of the areas of tissue loss. This homogeneous substance was found to accumulate first in the interlamellar spaces of the media causing disappearance of the muscle cells and of the very fine elastic fibers, and later of the collagen fibrils. When the changes became sufficiently extensive, fusion of adjacent clefts with destruction of the intervening elastic lamellas occurred. These developments were slowly progressive and were succeeded regularly by regenerative foci composed chiefly of smooth muscle cells with but sparse elastic and collagen fibril formation. Despite the often striking reparative attempts, the new-formed media seemed to be of inferior quality, and it in turn appeared predisposed to further degenerative mucoid and cystic changes. Cellina [44] had also studied these mucoid areas but expressed the belief that the primary injury concerned the medial elastic lamellas, resulting in disintegration, independent of muscle changes, and that ultimately cysts formed, filled with basophilic substance; he considered this mucoid as derived from a process of dedifferentiation of the elastic tissue. In another publication [45] he described widely distributed longitudinal band-shaped acellular focal areas of necrosis occurring in the inner aortic media of the elderly person unassociated with changes of the vasa vasorum. These appeared somewhat similar to the sparsely occurring areas described by Erdheim but were larger and more widely distributed, exhibited no tendency toward healing or formation of a substitute tissue and were of no significance, in his opinion, in producing vascular tears. It was felt that they probably represented phenomena of aging or an advanced degree of the cell dropping noted in the senescent aorta. Erdheim pointed out that one of the more prominent physiologic effects which aortic medial damage and muscle cell degeneration exerted on the circulation was due to an alteration of the "balloon" function of the vessel; in other words, because of the paucity of regenerated elastic fibers in the repaired zones, it was not possible for each dilatation of the aorta synchronous with systole to be followed by the elastic recoil of the wall necessary for the

44. Cellina, M.: Arch. ital. di anat. e istol. pat. **2**:1105, 1931.
45. Cellina, M.: Virchows Arch. f. path. Anat. **280**:65, 1931.

forward propulsion of the column of blood during diastole. Such an effect on circulation was not estimated to be great, however, in view of the physical capacity of patients in late life harboring such vessel changes. Indeed, it appeared to be of far less importance ultimately than the small amount of fibrous connective tissue repair in the damaged aortic media which permitted overstretching and actual tearing of the wall of the vessel.

Klotz and Simpson found patchy acellular areas of necrosis in each of 5 cases of spontaneous rupture of the aorta and noted that in these foci the muscle fibers were more severely damaged than the elastic tissue. Occasionally the debris of necrotic tissue remained in situ, but in other areas dissolution was effected by a humoral route without the cellular participation noted in previous cases. A colloid-like substance filling small clefts and cystlike spaces was described. Moritz,[27] Levinson,[46] Neuburger,[47] Wolff,[48] Narr and Wells,[49] Roberts,[50] Schattenberg and Ziskind,[51] Rottino,[52] Glendy, Castleman and White,[53] Kountz and Hempelmann,[54] and others, all have reported the presence of focal necrosis with mucoid accumulation in the media unaccompanied by inflammatory cell exudation and emphasized the importance of this condition in relation to dissection of the coats. Wolff found the medial changes most severe at the site of the tear in 5 of 6 cases examined, although they were also present in far removed zones as well as in other large arteries. He considered loss of elastic tissue the most striking feature of the medial damage in all cases. Weise [55] examined the aorta in 120 autopsies in which no noteworthy macroscopic changes were evident and found well marked zones of medionecrosis in 9. These areas involved the inner half of the media and favored the ascending aorta. The elastic fibers showed more extensive damage than the muscle or connective tissue elements, and mucoid substance was absent in the necrotic zones, as were evidences of tissue repair. A primary damage to the elastic elements of the vessel was also postulated here by Weise.

Rottino was able to demonstrate noninflammatory medial lesions of several types on examining the aorta in 12 selected cases of dissecting aneurysm. The lesions included focal loss of muscle with crowding

46. Levinson, B.: Virchows Arch. f. path. Anat. **282**:1, 1931.
47. Neuburger, K.: Ztschr. f. Kreislaufforsch. **24**:169, 1932.
48. Wolff, K.: Virchows Arch. f. path. Anat. **289**:1, 1933.
49. Narr, F. C., and Wells, A. H.: Am. Heart J. **8**:834, 1933.
50. Roberts, J. T.: Am. Heart J. **18**:188, 1939.
51. Schattenberg, H. J., and Ziskind, I.: J. Lab. & Clin. Med. **24**:264, 1939.
52. Rottino, A.: Arch. Path. **28**:1, 1939.
53. Glendy, B. E.; Castleman, B., and White, P. D.: Am. Heart J. **13**:129, 1937.
54. Kountz, W. B., and Hempelmann, L. H.: Am. Heart J. **20**:599, 1940.
55. Weise, W.: Beitr. z. path. Anat. u. z. allg. Path. **93**:238, 1934.

of the elastic lamina; loss of muscle with accompanying degeneration of elastic and collagenous fibers which progressed to mucoid cyst formation in some cases; scattered foci of muscle cells in areas devoid of elastic lamina, lying free in mucoid material or enmeshed loosely by collagenous tissue; occasional loosely constructed small fibrous scars, rarely containing vessels. In 11 of the cases the medial degenerative changes were confined exclusively to the ascending aorta and arch, an observation which is in accord with that in the majority of cases exhibiting cystic medionecrosis. Progression of the lesion from simple muscle loss to eventual cyst formation could be traced easily in 6 cases. This author was unable, however, to establish definitely that inception of the cyst formation followed overproduction of mucoid material in single interlamellar spaces, as maintained by Erdheim. Brouardel and Vibert [56] noted a complete degeneration of the medial coat in the aorta of a 20 year old man who died of a spontaneous rupture of this vessel. The media was described as constituted of a granular substance without structure containing some rare muscle cells but no trace of elastic tissue. The intima was thin but without alteration. In Wolff's [22] previously mentioned case, large disseminated foci of elastic lamella degeneration and destruction in the media of the aorta of a 12 day old infant was accompanied by mucoid accumulation and some damage to the collagen fibers and muscle cells. There was a striking absence of cellular inflammatory response or tissue regeneration in the involved zones. Fresh hemorrhages in some of these areas had not progressed to actual dissection of the medial coat.

NATURE OF THE MEDIAL MUCOID CHANGES

While mucoid degenerative areas of the media have been reported as the anatomic basis for aortic rupture in young people, older persons are far more commonly affected by this process, which may be present without formation of a dissecting aneurysm. Indeed, its role in initiating the latter condition appears to rest largely on a quantitative basis, and the accumulation of this substance may proceed to cyst formation without causing rupture of the coats.[57] Björling [58] described in detail the presence of a mucoid connective tissue in the intimal and medial coats of the normal as well as the pathologic aorta. In the former it was easily demonstrable as a red-staining fine irregular cotton fiber–like network when treated with Unna's polychrome methylene blue and differentiated in a mixture of aniline oil and alum. He pointed out that this tissue was not a forerunner of collagenous connective tissue fibers.

56. Brouardel, P., and Vibert, C.: Ann. d'hyg. **27**:451, 1892.
57. Rottino, A.: Am. Heart J. **19**:330, 1940.
58. Björling, E.: Virchows Arch. f. path. Anat. **205**:71, 1911.

In atherosclerosis and syphilis of the aorta, mucoid tissue was particularly abundant, and its increase was probably proportionate, he said, to the reduction of elastic and muscle fibers.

Schultz [59] confirmed the presence of mucoid tissue in the large arteries, less often in the veins, by using cresyl violet stain and differentiating with acetic acid. The greatest distribution of such tissue was found in the inner coats, and it increased with age. He found the interstitial ground substance to be the site of its earliest appearance, particularly in the vicinity of the elastic fibers, as noted before by Orsós in his description of the normal media. Finding a similar staining affinity on the part of the ground substance of the cornea and in cartilaginous tissue, Schultz suggested a possible relationship of this material to chondroitin-sulfuric acid. This so-called chromotropic substance in the arteries also displayed a definite affinity for fat and appeared to be a forerunner of further degenerative processes.

Costa [60] examined 50 human embryos from 1 to 3 months of age, as well as fetuses of varying ages, and compared the vessels with a control group of vessels taken from young infants. He found the arterial walls of the embryos rich in mucoid substance. This is visible in the first month of intrauterine life. Closer study of this substance showed that it checked histochemically, morphologically and histogenetically with the metachromatic substance of the embryonal mesenchyme. In general, with further development it was found to disappear from the walls of arteries of the elastic type and to persist in those of the muscular type. Chromotropic substance was not found after birth in the ductus Botalli or the umbilical artery. Further studies on 15 arteries from adults with hypoplasia of the elastica or muscle-elastica without syphilis or arteriosclerosis showed a diffuse arrangement of chromotropic substance throughout the media, in which it filled the spaces between the elastic fibers in a delicate net extending from one elastic fiber to another. He found no support for the belief that the chromotropic substance was a degeneration product or that it was dependent on the elastic fibers. It was more likely, he said, that its presence could be explained by a developmental disturbance. Its association with other vascular anomalies, such as coarctation of the aorta, as noted by Abbott and Hamilton and by Harrison,[61] or with other associated developmental anomalies, as in one of Neuburger's cases, would seem to favor this view. In Wolff's case of elastic and collagen fiber loss in the medial coat with mucoid changes in a 12 day old infant there was associated cardiac hypertrophy, widening of the aortic and

59. Schultz, A.: Ergebn. d. allg. Path. u. path. Anat. **22**:207, 1927.
60. Costa, A.: Ztschr. f. Kreislaufforsch. **23**:715, 1931.
61. Harrison, F. F.: Arch. Path. **27**:742, 1939.

pulmonic valve cusps, and other somatic defects. This author, however, did not accept the vascular lesion as a developmental anomaly in view of the demonstrable progressive degenerative elastic tissue changes and absence of damage to the elastic tissue of all other vessels and organs. Moritz, commenting on the increase of mucoid substance in the aorta of elderly persons, considered it an aging process.

Pathologic accumulation of chromotropic substances within the walls of large arteries then appears to take place in two distinct ways. It may result from overproduction of normally present mucoid interlamellar ground substance with encroachment on and replacement of adjacent muscle, elastic and collagenous elements. This has been thought to represent either a developmental disturbance or an accentuation of physiologic aging, although other stimulating influences must be considered. In another group of cases chromotropic substances appear to accumulate following primary degeneration or necrosis of the muscle, elastic or other elements in the vessel wall. There the material is in the nature of a "filling substance" replacing tissue elements initially destroyed by some other means.

ALTERATION IN THE GROSS APPEARANCE OF THE AORTA

Apart from the hematoma formation within the vessel walls in dissecting aneurysm, striking macroscopic changes may occur in adjacent portions of the aortic wall not involved in the dissection. Erdheim described slitlike gray scars or defects arranged somewhat in parallel and visible beneath a thin, smooth intima marking the site of medial damage. Niehaus and Wright [62] pointed out deep longitudinal grooves of the ascending and transverse portions of the aortic arch, causing narrowing of the isthmus. These were apparently formed by a decrease of substance in the aortic wall and corresponded to the medial defects.

Rottino [63] recently described the unusual case of a 76 year old woman whose ascending aorta was transformed into a diffusely dilated, nonelastic, thin-walled sac which ended abruptly in a moderately dilated arch. The intima of the sac was thick, gray and wrinkled, studded with numerous pearly plaques presenting the tree bark surface often seen in syphilis and diagnosed as such grossly. Microscopic sections, however, showed extensive foci of medial degeneration and necrosis but none of the changes of syphilis. Gouley and Anderson [64] also reported 6 cases of dissecting aneurysm in which during life there had been signs and symptoms of aortic regurgitation, with death attributable to long-standing heart failure rather than to rupture of the aneurysm. In 3

62. Niehaus, F. W., and Wright, W. D.: J. Lab. & Clin. Med. **26**:1248, 1941.
63. Rottino, A.: Arch. Path. **27**:320, 1939.
64. Gouley, B. A., and Anderson, E.: Ann. Int. Med. **14**:978, 1940.

of these cases at autopsy there was presented the gross appearance of syphilitic aortitis, i. e., a fine nodularity and scarring along the inner surface of the vessel, yet in no case could this be substantiated microscopically. Reexamination of the specimens showed the suggestive scarring confined to the region of new channel formation within the wall and due to irregular fibrosis and infolding of the new subendothelial fibroelastic tissue, apparently a part of an endothelialization process.

SEPARATION OF THE COATS AND ORIGIN OF THE INTRAMURAL HEMORRHAGE

The progression or coalition of any of the various types of degenerative medial lesions may eventually result in primary separation of this coat. The initial site of such dissociation is soon obscured if dissection proceeds to any extent. From the accumulated evidence it appears conclusive that the path of dissection is determined by the location and the extent of the medial lesion. This most frequently concerns the outer layer of the media, beginning in the ascending arm or arch of the aorta. The pressure of the blood within the aortic lumen and the rhythmic changes of the caliber of the latter favor progression of the hematoma once it has formed between the weakened layers. The great majority of cases present a transverse tear of the intimal coat in the ascending aorta, particularly at a point a short distance above the aortic valve cusps.[65] Frei noted that this occurred in 153 of 275 cases reviewed. The consistent presence of such a lesion has apparently been the basis for the assumption that this site is the starting point for seepage of blood between the coats; less frequently an atherosclerotic ulcer of the intimal coat has been demonstrated as the weakened spot which permits access to the circulating blood. Closer inspection of these suppositions indicates that the frequency with which such events occur has been considerably overestimated. Tyson's[66] experience of finding no intimal tear in 3 of the 5 cases of dissecting aneurysm he examined is corroborated by other observations in the literature. The presence of small hemorrhages originating from the vasa vasorum in an area of prominent medial destruction has already been mentioned in Moriani's case, in which the overlying intima was not perforated. Fahr[67] mentioned cylindric and even saccular outpouching of the vessel wall in the absence of intimal tearing. Krukenberg and Babes and Mironescu emphasized the importance of medial lesions independent of breaks in the intimal lining in producing dissection of the coats. Hamburger and

65. Weiss, S.: M. Clin. North America **18**:1117, 1935.

66. Tyson, M. D.: Am. J. Path. **7**:581, 1931.

67. Fahr, T., in Aschoff, L.: Pathologische Anatomie, ed. 8, Jena, Gustav Fischer, 1936, vol. 2.

Ferris [68] were unable to demonstrate intimal tears in 2 of their cases and Reisinger [69] reported on a massive aortic dissection without an intimal rupture. According to Winternitz,[70] there can be no doubt that the dissecting aneurysm arises from hemorrhage within the vessel wall and does not have its origin in a tear of the innermost surface.

Particularly interesting in regard to the extent of medial dissection possible without tearing of the intima is the case of Whitman and Stein.[71] They found the aorta of a 76 year old woman to be the site of an aneurysmal sac extending from the base of the heart to within 10 cm. of the iliac bifurcation. The sac was obviously the result of fusion of small degenerative clefts within the media and contained only lymph, with no evidence of either recent or old hemorrhages. The unbroken intima in the vicinity of the sac showed only slight to moderate atherosclerotic changes, which did not involve the media. It has also been observed that atherosclerotic ulcers are of rather uncommon occurrence in the thoracic aorta, where dissection most frequently takes place. When dissection does occur about such an ulcer, it usually involves the abdominal portion and is very limited in extent. Willius and Cragg,[72] studying the relationship between ulcerating atheromatous abscesses and dissecting aneurysm, found the muscular and elastic structures of the media destroyed in the locality of the atheromatous lesion while the areas immediately beyond presented little or none of the degenerative changes found in extensive dissections. Gallavardin and Gravier [73] reported on the case of a man 57 years old whose aorta showed a rather deep transverse laceration of the intima just above the valve cusps while no dissection of the coats was present. Microscopically, the elastic fibers were ruptured and destroyed at the site of the tear, with marked sclerosis of the base. Lateral to the tear, the elastic fibers of the media appeared relatively intact. It appears probable in view of the prerequisite medial changes that bleeding and the formation of intramural hematoma originate very often from the vasa vasorum within the vicinity of the destruction of tissue and, aided by circulatory stress, proceed along the path of least resistance. Such a mechanism obviously points to the frequent transverse supravalvular tears as the site of emergence rather than of entrance. They are similar to those seen penetrating the adventitia and discharging into the pericardial sac, pleura or elsewhere. Similarly, the weakening of the intimal coat by

68. Hamburger, M., and Ferris, E. B.: Am. Heart J. **16**:1, 1938.
69. Reisinger, J. A.: Arch. Int. Med. **65**:1097, 1940.
70. Winternitz, M. C.: Am. Heart J. **14**:480, 1937.
71. Whitman, R. G., and Stein, H. B.: J. M. Research **44**:579, 1924.
72. Willius, F. A., and Cragg, R. W.: Proc. Staff Meet., Mayo Clin. **16**:41, 1941.
73. Gallavardin, L., and Gravier, L.: Paris méd. **12**:23, 1922.

an atheromatous plaque would act as a locus minoris resistentiae for a secondary opening rather than for a primary tear when extensive dissection occurs. After reviewing over 300 cases of dissecting aneurysm, Shennan [74] stated that he found the number in which the process began in an atheromatous ulcer to be almost negligible. Klotz and Simpson concurred in minimizing the part played by alterations of the intima in localizing or causing a dissection. They stated that only in those rare instances in which an atheromatous ulcer by chance lay immediately above the medial lesion would it be of importance.

SITE OF PREDILECTION FOR INTIMAL TEARING

The explanation finding most general acceptance for the frequent occurrence of a transverse tear of the intima a short distance above the aortic valve cusps is that offered by Rindfleisch.[75] He expressed the belief that the pulmonary artery arising from the middle of the heart's base served as the principal support of the heart and aorta, ramifying widely in the lungs and broadening its area of support, following its short bifurcation in the mediastinum. He was constantly able to demonstrate the presence after middle life of bandlike thickenings of the pericardium passing from the pulmonary artery to the ascending aorta a short distance above the valve cusps and acting as clamps on this vessel, or vincula aortae. These he claimed determined the site of preliminary tearing of the aorta when the arch was stretched, particularly under the influence of an enlarged heart. This area of immobilization of the aorta was also strengthened by the obliterated ductus arteriosus, which served further to unite both vessels. The portions of the aorta about these zones of anchorage being relatively mobile, rupture would tend to occur at the sites of fixation.

Löffler's [76] explanation appears significant. He pointed out that when the aortic valves close, the abrupt recoil during diastole must cause a longtitudinal stretching of the ascending aorta, forcibly driving the first portion of the aorta downward and away from the transverse portion and putting an added strain at this point. Oppenheim, on the other hand, expressed the belief that the bursting tension of a vessel is in direct proportion to its radius; the smaller vessels were therefore capable of withstanding high pressure, while the ascending aorta, because of its greater circumference, could withstand the least. Shennan has brought out some interesting observations on the multiplicity of intimal tears. In analyzing 218 cases of recent dissecting aneurysm, he found multiple splits of the inner coats twenty-four times, or in 11 per cent,

74. Shennan, T.: J. Path. & Bact. **35**:161, 1932.
75. Rindfleisch, E.: Virchows Arch. f. path. Anat. **131**:374, 1893; **96**:302, 1884.
76. Löffler, W.: Cor.-Bl. f. schweiz. Aerzte **48**:1185, 1918.

and remarked that it is not always easy to determine which is the primary rupture, whether there has been more than one primary rupture or whether one or the other tear represents a reentrance rupture. In 79 cases of old dissecting aneurysm, multiple primary ruptures were noted eight times, or in 10 per cent of the cases. Separate dissections were found in 5 cases of recent dissecting aneurysm. If one assumes that hematoma formation within the coats of the thoracic aorta precedes damage to the intima, it seems likely that splitting of the latter would take place at the site of relative immobilization in the ascending aorta where pressure and strain are highest and that multiple tearing would more often be influenced by other local factors.

COURSE OF THE DISSECTION

In those cases in which the initial separation of the coats does not terminate abruptly with fatal hemorrhage, the subsequent course of the dissection varies considerably. It may be limited to a few centimeters on one side of the base of the aorta or may proceed to complete separation of the entire circumference throughout the length of the vessel; again it may involve not only the thoracic and abdominal aorta but also the large vessels emerging from both portions and may extend caudally as far as the lower border of both popliteal arteries, as noted by Gardner, Galbraith and Hardwick [77] in a 15 year old boy. In extensive dissections, the relatively small emerging intercostal and lumbar vessels are frequently torn across, and this sometimes interferes with circulation in the spinal cord, giving rise to neurologic disturbances [78]; or dissection may involve the larger emerging trunks supplying the gastroenteric canal, or may involve the renal arteries, thus giving rise to urologic symptoms as observed by Buckley.[79] In a large dissection the extension of the hematoma within the aortic coats proceeds almost entirely in a centrifugal direction propelled by and following the column of circulating blood within the vessel's lumen. Occasionally, however, the blood has been described as dissecting in a centripetal direction.

Hanser [80] reported in one of his cases a 2 by 1 cm. angular primary tear of the intima low down in the thoracic aorta. The blood extended upward between the intima and the media widening its circumference in the aortic arch where it reentered the lumen through a pinhead-sized opening above the valve cusps. Shennan also described a case in which an atheromatous patch one-half inch below the left subclavian artery was

77. Gardner, E.; Galbraith, A. J., and Hardwick, S. W.: Lancet **2**:1019, 1939.

78. Freistadt, K.: Virchows Arch. f. path. Anat. **237**:63, 1922. Tuohy, E. L.; Boman, P. G., and Berdez, G. L.: Am. Heart J. **22**:305, 1941.

79. Buckley, T. I.: J. Urol. **44**:816, 1940.

80. Hanser, A.: Deutsches Arch. f. klin. Med. **152**:61, 1926.

the site of dissection, the blood forcing its way backward as far as the heart and there involving about two thirds of the circumference of the aorta. Other observations in the literature corroborate such a centripetal course of the hematoma. Some of these, however, consist solely in the finding of clotted blood within the wall at varying distances upstream from the so-called primary intimal tear. Some progression of an intramural hematoma subject to constant pounding of the circulating blood can be visualized in either direction, provided medial degenerative or necrotic changes are well enough advanced to admit easy separation of the coats. However, it is hypothetic to assume that the dissection has proceeded centripetally merely because the hematoma extends a considerable distance above the intimal rip. Studies on the histologic age, as evidenced by the degree of organization present in sections from various portions of the dissecting hematoma, may throw further light on this point.

ULTIMATE FATE OF THE DISSECTED AORTA

The end result of dissection of the coats apparently depends on the individual tissue response to the extravasated blood as well as on the extent and the speed of the dissection. A second perforation of the intima some distance peripheral to the usual site of the tear in the aortic arch may ultimately reestablish circulation by organization and recanalization of the mass and the formation of a double-barreled aorta. Peery [81] found about 80 cases reported in the literature in which such an arterial reperforation was present. Weiss, Kinney and Maher [82] estimated that healing is likely to occur in approximately 10 per cent of the cases of total dissection of the aorta. In reporting 3 cases of "healed" dissecting aneurysm they stated that endothelialization had taken place and atherosclerosis developed over the internal surfaces of the new aortic channels in each case. In 1 instance the endothelialization and mild atherosclerosis of the new channel occurred within twenty-three months. Another exhibited calcareous plaques. With adequate intramural circulation so established, life may continue for a number of years. Hall [83] reported the development of a dissecting aneurysm in a 17 year old youth following a foot race. The patient lived fifteen years after this episode and was able to compete in strenuous athletic events. Graham's [84] patient lived more than thirty years after suffering an internal injury believed responsible for the development of the dissecting aneurysm. What is more, the aneurysm was found at autopsy to extend the whole length of the abdominal and thoracic aorta and to involve over one-half the circumference of the vessel.

81. Peery, T. M.: Arch. Path. **21**:647, 1936.
82. Weiss, S.; Kinney, T. D., and Maher, M. M.: Am. J. M. Sc. **200**:192, 1940.
83. Hall, E.: Arch. Path. **2**:41, 1926.
84. Graham, J. E.: Am. J. M. Sc. **91**:155, 1886.

On the other hand, in analyzing 143 recent cases of sheathlike dissection collected from the literature in which data on survival were obtainable, Shennan found that in 84, or 58 per cent, death occurred within twenty-four hours, and in 38, or 26 per cent, in from one day to one week; 11 patients, or 7 per cent, survived eight days to five weeks. No correlation between the position, the length or the direction of the tear, on one hand, and either the distance of the subsequent dissection or the period of survival, on the other, was permissible from the evidence collected. Frei found perforation through the outer aortic coat in 189 of 273 consecutive cases of dissection which he studied; in 139 the perforation communicated with the pericardial sac.

The term "healed," as it is commonly applied to cases in which a double perforation with recanalization takes place, is obviously a physiologic one. A true healing of the dissected coats through complete repair of the dissected portion by organization of the hematoma and obliteration of the channel may also occur and has been described in detail by Shennan.[74] In his case a 64 year old man exhibited recurrent hemorrhages into and along the walls of the aorta. One of these in the arch and descending aorta had occurred about eight months prior to death and had become organized into a dense layer of vascular connective tissue containing pigment-laden cells which abutted on the media. Another occurring two months before death had dissected along the outer layer of the media beneath the adventitia and had ruptured the inner coats opposite the ligamentum arteriosum but was completely organized and the sac obliterated. Shortly before death a third rupture had occurred in the right posterior wall in the region of the ligamentum arteriosum with hemorrhage into the media internal to the previously organized layer. This had caused an inward bulging of the upper dissected aorta with partial stenosis and a resulting increase of intra-aortic pressure leading to a fourth proximal rupture into the pericardial sac and to fatal cardiac tamponade. Frothingham, Sanderson and Hazard[85] described healed dissecting aneurysms appearing as prominent diamond-shaped depressions in the abdominal aorta and left common iliac arteries, associated with a recent dissection in the aortic arch which had led to fatal intrapericardial hemorrhage.

ETIOLOGY

No single causative agent or clinical disease process can at present be demonstrated as consistently producing the morbid changes leading to the formation of a dissecting aneurysm. In a small percentage of cases developmental errors of the formation of the aortic media, revealed by a deficiency or a failure of one or more tissue elements, have pre-

85. Frothingham, C.; Sanderson, E., and Hazard, J. B.: Tr. A. Am. Physicians **54**:333, 1939.

disposed the vessel to dissection. Most commonly in these cases there are associated vascular defects, such as coarctation of the aorta, anomalies of the valve cusps or various somatic changes. Boyd and Werblow [86] described definite thinning of the aortic media associated with aortic coarctation in their case of dissecting aneurysm. The observations of Abbott and Hamilton in their cases of coarctation have been mentioned. St. George,[87] in his report of aortic rupture in a 45 year old man, described a diffusely dilated thin-walled aorta in which the elastic tissue of the media was considerably diminished but associated anomalies were absent. A developmental basis for the defect in this case is questionable. In Brouardel and Vibert's unusual case of rupture resulting from a medial defect, the condition was thought to be either a congenital maldevelopment or the sequel of an attack of typhoid fever in childhood. Marine [88] mentioned deficiency of muscle fibers and elastic tissue in the arterial wall and rupture of the wall in status lymphaticus.

MacCallum [89] reported that intimal arteriosclerosis plays a role by permitting unusually easy separation of the coats of the aorta, leading to dissection, but this does not appear to be borne out by the majority of observations, as has been noted in the description of the medial lesions. Tyson found intimal sclerosis of the vasa vasorum constantly present in his cases and suggested a nutritional effect on the aortic media. Lund [90] also found the vasa vasorum sparse and sclerotic in the aorta of a 20 year old woman which exhibited advanced intimal sclerosis, marked medial degeneration and a large dissecting aneurysm. Such changes of the vasa vasorum associated with a medial lesion have been exceptional.

The role of hypertension appears to be a subordinate one, though it definitely influences the course of dissection once the prerequisite medial changes are established. Shennan noted that in 163 of 218 cases of recent dissecting aneurysm, indications of the condition of the heart were given at autopsy. In 131, or 80 per cent, the left ventricle was described as hypertrophied, with or without dilatation. In the remaining 32 cases, or 20 per cent, there was evidence that high blood pressure, at least a permanent rise, was not an invariable factor. In 18 of these 32 cases, the heart was found normal or not hypertrophied. McGeachy and Paullin collected 127 cases from the literature in which clinical data were available and noted that a history of hypertension was given in 60 cases and exertion at the time of onset of the dissection was mentioned in 33. While these and the preceding figures indicate a high incidence of associated hypertension in cases of dissecting aneurysm, it is not

86. Boyd, L. J., and Werblow, S. C.: Ann. Int. Med. **11**:845, 1937.
87. St. George, A. V.: Am. J. Syph. **4**:702, 1920.
88. Marine, D.: Arch. Path. **5**:661, 1928.
89. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **20**:9, 1909.
90. Lund, H.: Arch. Path. **15**:162, 1933.

clear how such a state would produce medial damage, as has been suggested by Babes and Mironescu and more recently by Shennan. Bohnen [91] called attention to the factor of hypertension during pregnancy, citing a rise of blood pressure of 60 to 100 mm. of mercury at the height of a labor pain, and reported a case of rupture of the aorta in a 26 year old woman during labor. He collected 6 similar cases from the literature but did not give adequate consideration to the microscopic appearance of the aorta. That severe damage of this vessel may have been the determining factor is suggested by the cases of aortic rupture in late pregnancy reported by Göbel [92] and Milew,[93] in which medial degenerative changes with mucoid accumulations were prominent microscopically. Wegelin [94] found dissecting aneurysms in the inferior thyroid arteries of a young woman who died of eclampsia, and expressed the belief that there was some association between them and thyroid disease, which was present Krukenberg, on reviewing this case, suggested that the medial necrosis within the vessels may have been caused by circulating toxins, such as frequently cause damage of the liver in eclampsia. Wolff and Weise both stressed a primary or essential damage to the elastic tissue of the aorta and expressed the belief that this follows repeated minimal traumatization. This may take the form of hypertension with increased pressure within the vessel or may follow overdistention subsequent to organic changes in other portions of the vascular tree. These theories support the observation of Hammer [95] on the local traumatic fraying of elastic fibers within the media, though a possible associated toxic action is not denied.

The role played by syphilis in dissecting aneurysm has been considerably disputed. Gsell asserted that there was a syphilitic toxin capable of producing muscle necrosis in the aorta unassociated with cellular infiltration or adventitial reaction but offered no concrete evidence for the assertion. On the other hand, he pointed out that muscle cell necrosis in the aorta may occur following arsphenamine therapy for syphilis and conceivably the damage might be sufficiently severe to permit dissection of the coats. Of 3 cases of dissecting aneurysm reported by Lifvendahl,[96] all showed hypertension, renal arteriosclerosis and syphilitic mesaortitis, always most marked at the site of the intimal tear. In 1 case rheumatic disease could not be excluded. Holland and Bayley found syphilitic aortic changes in 5 of the 19 cases of dissecting aneurysm they studied, and Peery [97] found

91. Bohnen, P.: Zentralbl. f. Gynäk. **51**:2398, 1927.
92. Göbel, A.: Zentralbl. f. Gynäk. **60**:38, 1936.
93. Milew, L.: Zentralbl. f. Gynäk. **60**:2912, 1936.
94. Wegelin, C.: Berl. klin. Wchnschr. **46**:2094, 1909.
95. Hammer, E.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:541, 1932.
96. Lifvendahl, R. A.: Arch. Path. **8**:200, 1929.
97. Peery, T. M.: Am. Heart J. **12**:650, 1936.

definite, well marked syphilitic medial lesions in 1 of 5 cases occurring in Negroes. Loeschke's [98] case of dissecting aneurysm showed gumma formation penetrating the wall from the adventitia and preparing the coat for dissection. This author reviewed the literature on those cases in which syphilis was associated with dissecting aneurysm and concluded that in general syphilis does not favor but rather hinders the development of dissection. Klotz also maintained that the very nature of the syphilitic process, with the development of connective tissue bands in the aorta, precludes the splitting of the wall into the lamellas seen in dissecting aneurysm.

The lesions of rheumatic fever in the aorta have been carefully studied by Pappenheimer and Von Glahn,[99] Perla and Deutch [100] and others. The late effects of these changes may in rare cases be sufficiently severe to cause dissection of the aortic coats, as reported by Gray [101] in a 39 year old man with a long history of rheumatic fever.

Various toxins have also been mentioned as contributing to medial necrosis. Gsell suggested a deleterious effect exerted by uremic toxins and, in 1 case, by nicotine. Furno [102] also suggested nicotine poisoning. Boveri [103] stated that atherosclerotic aortic lesions could be produced by feeding rabbits tobacco. Adler and Hensel [104] further claimed that intravenous injections of dilute solutions of nicotine produced lesions of two types in the rabbit's aorta. One was characterized by varying degrees of aneurysmal dilatation following an attack on the inner medial muscle cells which caused necrosis, calcification and secondary damage to the elastic fibers. The other type showed plaque formation and unevenness of the lining surface following primary necrosis of the inner medial muscle cells. No changes were noted in the vasa vasorum.

Duff [105] succeeded in producing medial necrosis in animals with diphtheria toxin. Vascular changes in man following various infectious diseases have also been mentioned by Wiesel and by Stoerk and Epstein. The presence of such regularly appearing, marked vascular changes, presumably the result of circulating toxins, could not be substantiated by Scharpff [106] in his studies of acute infectious disease, although he noted occasional slight degenerative changes in the peripheral vessels.

98. Loeschke, A.: Frankfurt. Ztschr. f. Path. **36**:56, 1928.
99. Pappenheimer, A. M., and Von Glahn, W. C.: J. M. Research **44**:489, 1924.
100. Perla, D., and Deutch, M.: Am. J. Path. **5**:45, 1929.
101. Gray, S. H., and Aitkin, L.: Arch. Path. **8**:451, 1929.
102. Furno, A.: Centralbl. f. allg. Path. u. path. Anat. **35**:188, 1924.
103. Boveri, P.: Deutsche med. Wchnschr. **32**:2085, 1906.
104. Adler, J., and Hensel, O.: Deutsche med. Wchnschr. **32**:1826, 1906.
105. Duff, G. L.: Arch. Path. **13**:543, 1932.
106. Scharpff, A.: Frankfurt. Ztschr. f. Path. **2**:391, 1909.

The experimental effect of epinephrine on the aorta of the rabbit has long been known. Kulb [107] found a dissecting aneurysm in a rabbit's aorta following repeated injections of epinephrine over a period of sixty-two days. Erb [108] found the essential lesions to be medial muscle necrosis caused by direct action of the epinephrine, followed by calcification and elastic tissue degeneration with later thickening of the intima. Multiple saccular aneurysms were also found. Waterman [109] postulated three modes of action of epinephrine: increase of blood pressure; direct destruction of the medial muscle cells by chemical action; anemic necrosis following constriction of the vasa vasorum. Simultaneous application of amyl nitrate with epinephrine did not prevent the muscle necrosis.

Leary and Weiss [110] reported the formation of a dissecting aneurysm in a rabbit's aorta following the feeding of large doses of cholesterol; the animal lived three years after the feeding had been discontinued. The area of dissection occurred about a large atheromatous ulcer in the descending aorta. The authors concluded that the diffuse atherosclerotic process induced by cholesterol was the primary factor in the production of the aneurysm. Wolff,[22] commenting on the changes observed by Steinbiss [111] in the rabbit's aorta following maintenance on a protein-rich diet, suggested that a vitamin deficiency may bear some relation to the medial lesions observed in man.

In evaluating these lesions it seems important to recognize the variability of the rabbit's response to various substances. Schirokogoroff [112] called attention to the extremely individual response of rabbits to epinephrine, some showing marked lesions of the aorta in a week while others showed no change after six months. Kesten [113] noted an early incidence of spontaneous aortic medial degeneration in rabbits, and Bennecke [114] placed the incidence of vascular disease in a series of 400 untreated rabbits at 3 per cent. One animal presented a spontaneous dissecting aneurysm. Löwenthal [115] also found aortic tears occurring spontaneously in mice with medial necrosis. Working with dogs, Samson [116] found no changes in the aorta following repeated intravenous injections of epinephrine. These contradictory and confusing results of experiments on animals leave the causes of dissecting aneurysm poorly understood.

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107. Kulb: Arch. f. exper. Path. u. Pharmakol. **53**:140, 1905.
108. Erb, W., Jr.: Arch. f. exper. Path. u. Pharmakol. **53**:173, 1905.
109. Waterman, N.: Virchows Arch. f. path. Anat. **191**:202, 1908.
110. Leary, T., and Weiss, S.: Arch. Path. **29**:665, 1940.
111. Steinbiss, W.: Virchows Arch. f. path. Anat. **212**:152, 1913.
112. Schirokogoroff, J. J.: Virchows Arch. f. path. Anat. **191**:482, 1908.
113. Kesten, H. D.: Arch. Path. **20**:1, 1935.
114. Bennecke, A.: Virchows Arch. f. path. Anat. **191**:208, 1908.
115. Löwenthal, K.: Virchows Arch. f. path. Anat. **265**:424, 1927.
116. Samson, P. C.: Arch. Path. **13**:745, 1932.

SUMMARY

Few clearcut descriptions of dissecting aneurysm are present in the literature prior to the autopsy reports on this condition published in 1761 by Morgagni. At present the total number of cases reported in the literature is about 500. This includes 33 cases diagnosed before death and verified at autopsy.

The period of highest incidence is between the fourth and seventh decades of life. At one extreme of life is the reported case of dissecting aortic aneurysm with fatal rupture in a 14 month old boy. Another case, that of a 12 day old infant showing destructive medial lesions with focal hemorrhages in the aorta, appears to present the early development of a dissecting aneurysm. The oldest patient recorded exhibiting a dissecting aneurysm was a woman nearly 100 years of age.

Males are reported affected twice as often as females, though in the higher decades of life females appear to predominate slightly.

There is little supporting evidence for the assumption that a dissecting aneurysm may form in a healthy aorta when the intravascular pressure is greatly raised. Prerequisite for dissection is damage to the medial coat of the aorta. It may be of several types. Common to the great majority of instances is a process which is independent of changes in the vasa vasorum or the intimal coat of the aorta and which is unaccompanied by exudative cellular inflammatory changes. When reacting cells are present, they are usually few and appear to be secondary to degenerative lesions. There are various types of medial lesions: (1) primary degeneration of the elastic lamellas in the form of fatty metamorphosis, fragmentation or necrosis, with varying amounts of damage to the supporting collagenous or muscle fibers, with or without mucoid accumulations; (2) hyaline degeneration of the interlamellar connective tissue; (3) varying degrees of fatty degeneration and atrophy of muscle cells and surrounding structures; (4) nonexudative necrosis of muscle cells proceeding from simple nuclear dropping to extensive structureless homogenization of the muscle cells and the adjacent collagen and elastic fibers with or without mucoid accumulation; (5) primary overproduction of mucoid substance in the interlamellar ground substance with encroachment on the muscle, elastic and collagenous fibers and ultimate formation of cysts. Actual quantitative reduction of the medial tissue elements, linked with marked thinning of the wall of the vessel, may be associated with any of the preceding degenerative lesions. In cases in which pronounced mucoid accumulations with cyst formation are observed, these appear most often to involve the thoracic aorta, particularly the ascending portion of the arch.

Areas of destruction of medial tissue may be replaced by poorly vascularized collagen fiber scars or by regenerated muscle and elastic

tissue. Removal of necrotic tissue is effected through a humoral route. Some acellular zones of necrosis show no tendency toward replacement by a substitute tissue.

The mucoid or chromotropic substance described in the aortic media has been found to be similar to the metachromatic substance of embryonal mesenchyme. It is present in early embryonal life and is also found in small quantities in the normal adult aorta. Pathologic increases in this substance, preparing the way for a dissecting aneurysm, may result from overproduction of the normally present mucoid interlamellar ground substance, with encroachment on and replacement of adjacent muscle, elastic and collagenous elements. It may also accumulate after primary degeneration or necrosis of the muscle, elastic or adjacent supporting structures. Here the material appears to be in the nature of a "filling substance" replacing tissue destroyed by some other means. Its increase through primary overproduction of the interlamellar ground substance may be the result of a developmental disturbance or it may be part of an aging process. It possibly represents a reaction to some "toxic" stimulus.

Gross pathologic changes indistinguishable from those of syphilis may be present in cystic medionecrosis of the aorta.

The path of dissection of the aortic coats is determined by the location and the extent of the medial lesion. Intramural hematoma formation is frequently present without tearing of the intimal coat and takes its origin from damaged vasa vasorum. Dissection usually occurs between the outer medial layer and the adventitia. Elevation of intravascular pressure undoubtedly aids dissection once it has begun.

The frequent localization of a large intimal tear in the supravalvular portion of the aorta appears to be due to the relative immobilization of the vessel at this point, together with the marked physiologic strain and pressure acting on this area. The intimal splitting here may represent the site of emergence rather than that of origin of the hematoma. In large dissections separation of the coats proceeds almost entirely in a centrifugal course. Occasionally the blood has been described as dissecting centripetally.

While death usually occurs within a few hours or days from the onset of dissection of the aorta, because of perforation of the outer coat, the development of a second intimal opening with recanalization of the intramural mass is compatible with long life. Endothelialization of the new-formed channel may be followed by atherosclerotic changes and calcification. Rarely, true healing of the dissected area may take place by organization of the blood clot.

No single causative agent or morbid process can be demonstrated as consistently producing the vascular changes leading to dissecting aneurysm.

Notes and News

Appointments and Retirements.—Herman G. Weiskotten, professor of pathology and dean of Syracuse University College of Medicine, has been appointed secretary of the Council on Medical Education and Hospitals of the American Medical Association.

Lucius F. Badger, surgeon, United States Public Health Service, has become assistant director of the National Institute of Health.

Ralph E. Miller, associate professor of pathology, Dartmouth Medical School, Hanover, N. H., has been chosen president of the state board of health.

Arthur I. Kendall, professor of research bacteriology, Northwestern University school of Medicine, Chicago, will go on the retired list at the end of the present academic year.

John H. Mueller has been advanced from associate professor to professor of bacteriology and immunology in the Harvard Medical School, Boston.

Cancer Fellowships and Tumor Diagnosis.—The Finney-Howell Research Foundation, Baltimore, has awarded eight fellowships in cancer research. Applications for 1943 must be in the hands of the secretary of the foundation by Jan. 1, 1943.

Under the division of cancer control of the state department of health of Illinois, subsidized laboratory services for the free diagnosis of tumors have been established in four hospitals outside Chicago.

Awards.—Leo Loeb, emeritus professor of pathology at Washington University, St. Louis, was presented on March 3 with the Award of Merit and Gold Medal of the St. Louis Medical Society.

Society News.—The organization of the Rhode Island Society of Pathologists has been announced, with B. E. Clarke as president and L. Goodman as secretary-treasurer.

The next annual meeting of the American Association of Pathologists and Bacteriologists will be held in Chicago, April 1 and 2, 1943. The newly elected officers are Paul R. Cannon, president; Wiley D. Forbus, vice president; Howard T. Karsner, secretary; Alan R. Moritz, treasurer. Infectious granuloma, exclusive of tuberculosis and syphilis, will be the topic for the symposium at the 1943 meeting, with Wiley D. Forbus as referee. The Gold Headed Cane of the association was awarded to James Ewing.

Deaths.—R. W. Hegner, professor of protozoology and head of the department of medical zoology in the Johns Hopkins University, died on March 11, at the age of 62 years.

Lawrence J. Henderson, Abbott and James Lawrence professor of chemistry, Harvard Medical School, Boston, died on February 10, aged 63.

George S. Graham, professor of pathology in the school of medicine of the University of Alabama, died on May 2, at the age of 63.

Book Reviews

Neural Mechanisms in Poliomyelitis. Howard A. Howe, M.D., associate in anatomy, Johns Hopkins University, Baltimore, and David Bodian, Ph.D., M.D., assistant professor of anatomy, Western Reserve University, Cleveland. Pp. 234, with 39 plates. Price $3.50. New York: The Commonwealth Fund, 1942.

This book presents, with discussions, results obtained in experimental work on poliomyelitis during the past five years. It deals mainly with the virus-neuron relations in the disease. Thomas M. Rivers writes a succinct foreword, in which he emphasizes the need of research on poliomyelitis by the neurobiologic methods employed by the authors. Headings of chapters will give a good idea of the contents: The neurotropisms of the virus of poliomyelitis and the mechanism as well as the rate of the virus' spread in the peripheral nerves; the genesis of the cerebral lesions; the role of neurons in the spread of the virus in the central nervous system; the behavior of the virus in peripheral nerves and in non-nerve tissues; the portal of entry in the experimental animal (page 97) and in man (page 125); the lesions of early arrested and nonparalytic poliomyelitis; virus-refractory states in nerve cells; problems of immunity to poliomyelitis presented by second attacks. The last chapter contains an instructive summing up. The book is well written and well printed. The many black and white figures on the 39 plates illustrate satisfactorily the reactions to the virus under various conditions. The specialized technic of the well planned work the book describes has yielded significant results. It is shown, to mention only one point, that the virus of poliomyelitis is not only neurotropic but actually highly "neuronotropic" and that in the peripheral nerve it migrates along the axon, using, if necessary, roundabout ways to reach the nerve center. In some monkeys and especially in the chimpanzee, the virus can easily reach the central nervous system from the oral cavity or the intestinal tract. In the rhesus monkey, the animal most used so far in the experimental study of poliomyelitis, the virus takes the olfactory route. The evidence indicates strongly that different routes may be taken by the virus in different species, and in man the facts now at hand point to the oropharyngeal and gastrointestinal mucous membranes as portals of entry. The book will be of great value in the continued investigation of poliomyelitis.

Diseases of the Basal Ganglia. Research publications, Association for Research in Nervous and Mental Disease. Vol. 21. Cloth. Pp. xii + 719. Price $10. Baltimore: The Williams & Wilkins Company, 1942.

This book, composed of papers presented at the meetings of the Association for Research in Nervous and Mental Disease on Dec. 20 and 21, 1940 by twenty-nine outstanding investigators in the field of neurology, deals admirably with a difficult subject. Probably an inherent defect in this type of presentation of a narrow field is the too frequent occurrence of repetitious material. The book may be divided into three parts, one on the anatomy and the physiology, a second on the clinicopathologic features and a third on the treatment of diseases of the basal ganglions.

A historical summary, followed by a general review of the fiber connections of the basal ganglions, introduces the first section. The material in this portion of the book is clearly presented and contains much that is new. The present conception of the function of the basal ganglions appears to be undergoing radical changes, and if the unanimity in the conclusions of the different investigators may be taken as a criterion, the trend is toward a truer appreciation of the physiology of these structures. The argumentation of chapter 6 on the relation between the pyramidal and the extrapyramidal function is unduly long for the amount of new

data presented. The title of chapter 8, "Physiological Neuronography of the Cortico-Striatal Connections," seems inappropriate in view of the use of strychninization as the experimental method. This technic, which one of the authors has previously spoken of as the "setting on fire" of nervous structures, can hardly be called physiologic. This chapter, however, in spite of its pretentious title is a clear presentation of the excellent research by the late Prof. Dusser de Barenne and his associates.

The clinicopathologic section attempts the herculean task of correlating clinical and anatomic findings in disease of the basal ganglions. The chapter by Alexander, describing in detail the various pathologic conditions which give rise to athetoid or parkinsonian states, is probably the best in the book. His explanation of status marmoratus is ingenious and quite convincing.

The treatment of diseases of the basal ganglions is introduced by a review of the medical management, which is dispensed with in 22 pages, giving way to a 146 page discussion of the surgical treatment. The papers of Bucy, Meyers and Putnam are well documented and substantiate their thesis that surgical therapy has a definite place in the treatment of diseases of the basal ganglions. The report by Klemme, who has had much more experience than any one else with this form of therapy, is disappointing. It is so brief and indefinite that his statistics, which represent practically one half of the paper, cannot be critically evaluated. Even these data are confusing; for example, Klemme indicates in his table of results that in 58 cases there was "complete relief of tremor and other distressing symptoms with complete rehabilitation," but in his summary he states, ". . . thirty-nine patients have been completely relieved and rehabilitated without any clinical signs or symptoms." The operative mortality in Klemme's series was 17 per cent, but the neurologic morbidity (hemiplegias, etc.) is not given.

The book is an excellent presentation of the knowledge of the basal ganglions, one which is of interest to clinicians, neuroanatomists, neuropathologists and practitioners who wish to know the present day status of this subject.

Stanford University Publications, University Series, Medical Sciences. Volume IV. Number 2. Lane Medical Lectures: The Lymphatic System, Its Part in Regulating Composition and Volume of Tissue Fluid. Cecil K. Drinker, professor of physiology and dean of the School of Public Health, Harvard University. Pp. 101, with 39 figures. Price $2.25 (paper, $1.50). Stanford University, Calif.: Stanford University Press, 1942.

This book contains the twenty-eighth series of the Lane Medical Lectures, the first series of which was given in 1896. In his preface the author says: "I have tried my hand at developing the reasons why mammals have lymphatics; why the lymphatic system has been slowly turned from a casually organized accessory of the blood circulation into a physiological entity, complementing this first system and joining with it in the task of keeping the composition and volume of the mammalian tissue fluid at a steady normal level." This task is well done. Highly instructive accounts are given of the evolution of the mammalian circulation of the blood, the establishment of the capillary system and the appearance and elaboration of lymphatic vessels. The interdependence of the blood and lymph circulations is well illustrated by the results of recent experiments of the author on the relations of blood, lymph and tissue fluid in the heart and the lungs. The final lecture deals with the role of the lymphatics in the healing of wounds and in pulmonary fibrosis. The illustrations are helpful and in many cases of great historical interest. In 1910 Dr. R. H. Fitz gave a series of Lane Lectures, entitled "A Consideration of Some Features of the Lymphatic System," but unfortunately the knowledge and the beliefs then expressed cannot be contrasted with those now current because the Fitz lectures have not been found. Dr. Drinker's lectures form an attractive and valuable supplement to the recent book on the lymphatic system by himself and J. M. Yoffey (Lymphatics, Lymph and Lymph Tissue, Cambridge, Harvard University Press, 1941; reviewed, ARCH. PATH. **32**:885, 1941).

Books Received

ANOXIA, ITS EFFECT ON THE BODY. Edward J. Van Liere, Ph.D., M.D. Pp. 269. Price $3.00. Chicago: University of Chicago Press, 1942.

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